The effect of different types of nutrition on the gastrointestinal response of the preterm infant

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This thesis is presented for the degree of Doctor of Philosophy (Biochemistry) at The University of Western Australia Faculty of Science, School of Chemistry and Biochemistry

2015
Preface

The work presented in this thesis was supervised by Associate Professor Donna Geddes and Winthrop Professor Peter Hartmann from the School of Chemistry and Biochemistry and by Winthrop Professor Karen Simmer from the School of Paediatrics and Child Health. The Women and Infants Research Foundation, Subiaco financially supported my candidature and Medela AG, Baar, Switzerland, provided a top-up scholarship.

The work presented in this thesis is my own work except when stated. All data collection was conducted in the Special Care Nurseries at King Edward Memorial Hospital, Subiaco. The material presented in this thesis has not been presented in any other degree.

This thesis is presented as a series of scientific papers, of which two have been published (Chapters 2 and 3), with Chapter 4 recently accepted for publication after completion of minor revisions, and Chapter 5 submitted to a peer-reviewed journal. Parts of the thesis have been presented at scientific conferences in both poster and oral format (Publications on pages vi – vii). Permission has been granted by co-authors to include their work in this thesis (Statement of candidate contribution on pages viii – ix). To ensure consistency throughout the thesis, all published papers are identical to that submitted for publication with the exception of the formatting and numbering of tables, figures and references.
Abstract

Background

Delayed gastric emptying is a common complication in preterm infants and may indicate the onset of feeding intolerance or serious disease such as necrotizing enterocolitis. Feed composition and intragastric curding are thought to impact gastric emptying, and pasteurized donor human milk (PDHM) is used in the absence of mother’s own milk (MOM) although the effect of their differing compositions on gastric emptying is not known. A thorough examination of gastric emptying and curding in response to feeds of differing compositions is essential to the advancement of knowledge and management of preterm nutrition, yet there have been no validated methods available to achieve this.

Aims

In this study we aimed to validate a sonographic method of measuring stomach volume and identifying the echogenic characteristics of breastmilk feeds, and to determine intra-individual repeatability. We then evaluated the effects of feed composition on gastric emptying and curding in preterm infants, specifically with regard to unfortified and fortified mother’s own milk (MOM) and pasteurized donor human milk (PDHM).

Methods

To determine the accuracy of ultrasound in measuring stomach volume, we studied 24 stable preterm infants born <34 weeks gestation that were receiving breastmilk feeds via an intragastric tube. Infants were monitored during a single intragastric tube feed with two stomach ultrasound images and one antral cross-sectional area (ACSA) image recorded when 50, 75 and 100% of the feed was delivered. Image measurements were used to calculate ACSA and stomach volume using three different methods. Raw measurements, ACSA and volume calculations were tested for intra- and inter-rater agreement. Calculated stomach volumes and ACSA were compared to delivered feed volumes, with echogenic characteristics of
stomach images graded at each time point.

To determine the repeatability of sonographic stomach volume calculations and ratings of echogenicity and gastric curding 20 preterm infants were studied. Paired feeds of identical composition and volume (n=29) were monitored pre and post feed and every 30 minutes thereafter. Statistical comparisons of paired stomach volume calculations and agreement between echogenicity and curding ratings were made for each time point.

The influence of biochemical and energy concentrations as well as fortification on gastric emptying of MOM was studied in 25 preterm infants. Paired feeds of MOM +/- human milk fortifier (HMF) were monitored pre and post feed and at 30 minute intervals with two differing HMF compared; S-26 (casein and whey, n=8) and FM 85 (whey only, n=17). Influences of infant and feed characteristics were also explored. MOM samples from each feed were analysed to determine concentrations of total protein, casein, whey, lactose, fat, and energy. Fortified feed compositions were calculated using unfortified MOM and HMF biochemical and energy concentrations.

To investigate whether PDHM differed from MOM with respected to gastric emptying paired feeds of unfortified and FM 85 fortified PDHM (n=15) and MOM (n=17) were examined with ultrasound (gastric volume and curding) as previously described. The influence of biochemical and energy concentrations, HMF, infant and feed characteristics were again explored.

Results

A spheroid volume calculation provided a direct measure of preterm stomach volume that is repeatable (ICC=0.984, 0.976<ICC<0.989), reliable (ICC>0.99, CI=95%) and accurate to ±1 mL. Moderate levels of consistency were observed for ratings of echogenicity (κ=0.44) and curding (κ range 0.31 to 0.65) between feeds of the same volume and composition.

Higher MOM casein concentrations were associated with faster gastric emptying during feed delivery (p=0.007). When compared to unfortified MOM, FM 85 fortified feeds emptied more slowly both during feed delivery and over the postprandial period (p=0.002, p<0.001, respectively), while emptying of S-26 fortified feeds was similar
(p=0.23, p=0.78, respectively). Gastric emptying was slower for 2 hourly feeds compared to 3 hourly feeds (p=0.003) and in supine position compared to prone (p=0.001).

Biochemical concentrations differed significantly between MOM and PDHM both before and after fortification. Gastric emptying during feed delivery was faster for both MOM and PDHM feeds of higher casein, whey and lactose concentrations (all p<0.001) irrespective of fortification, while faster postprandial emptying was associated only with a higher casein concentration (p<0.009).

PDHM while having a different composition emptied more slowly than MOM, with retained feed proportions approximately 23% higher immediately post feed (p=0.026) and 15% postprandially (p=0.006), although final gastric residual volumes are comparable (MOM vs. PDHM; 2 hourly feeds 2.5±1.6 mL vs. 1.6±2.0 mL respectively, p=0.55; 3 hourly feeds 0.6±1.1 mL vs. 1.9±3.9 mL respectively, p=0.80). Echogenicity and curding of MOM and PDHM were similar and did not influence gastric transit. Slower gastric emptying of PDHM could not be explained by compositional differences either. Fortification of MOM and PDHM with FM 85 was associated with slower gastric emptying during feeding and postprandially (both p<0.001), while both small feed volumes and supine positioning of the infant were associated with slower postprandial emptying (p<0.001, p=0.002, respectively).

**Conclusion**

Ultrasound imaging has been validated as an accurate reliable method of measuring stomach volume in preterm infants. It is sensitive enough to detect differences in gastric emptying due to milk volume, composition, fortification, the type of milk fed and infant position and to characterize curding. Ultrasound has great potential as a bedside tool for the clinician to evaluate infants with gastrointestinal symptoms as well as to monitor new nutritional products and feeding strategies.
Acknowledgements

This journey would not have been possible without the support of my family, professors, colleagues and friends. Firstly I would like to express my gratitude to my supervisors Associate Professor Donna Geddes, Winthrop Professor Peter Hartmann and Winthrop Professor Karen Simmer. My deepest appreciation goes to my coordinating supervisor, Associate Professor Donna Geddes for your steadfast support and mentorship. Thank you for inspiring me, believing in me and for providing me with opportunities to grow both personally and academically. Winthrop Professor Peter Hartmann, thank you for your invaluable guidance, teaching and insights and also for the opportunities you have given me. Winthrop Professor Karen Simmer, thank you for your expertise and advice throughout my candidature.

The support of the Hartmann Human Lactation Research Group staff and students, both past and present, has been invaluable. Special thanks to Mrs Ruth Abbott for your ongoing kindness and assistance from the very beginning of this journey, to Ms Anna Hepworth for your statistical expertise and to Foteini, Vanessa, Lukas, Jackie, Ching, Zoya and Hazel for your friendship, advice and helpful discussions. The assistance provided by Dr Ben Hartmann, Mrs Tracey Sedgwick and my colleagues in the Special Care Nurseries at King Edward Memorial Hospital has been greatly appreciated. I am very thankful for the interest in my work and scholarships provided by Medela AG and the Women and Infants Research Foundation.

Finally, I would like to express my gratitude to my family and friends from whom I have received such generous support and understanding. I am forever grateful for my husband and best friend, Eugene, without whose love and practical, financial and emotional support I would not have completed this thesis. To Eugene and our beloved children Daniel, Sofie and Katia, thank you for encouraging me in my pursuits and inspiring me to follow my dreams. I dedicate this work to you.
Research Papers


Conference Abstracts


Seminars


Statement of candidate contribution

The author, Sharon Perrella, completed the majority of the work presented in this thesis. However other individuals contributed to each chapter and their subsequent publication or submission to a peer reviewed journal.

Chapter 1

The author reviewed the literature and wrote the chapter. Associate (A) Professor Donna Geddes, and Professor Peter Hartmann and Professor Karen Simmer reviewed the chapter and provided feedback.

Chapter 2

The author contributed to the design of this study, and contributed to data collection and data analysis while undertaking extensive training provided by A/Professor Donna Geddes. The author contributed to the statistical analysis and prepared the manuscript for publication. A/Professor Donna Geddes, Professor Peter Hartmann and Professor Karen Simmer were involved in the study design. Ms Anna Hepworth provided statistical support. All co-authors reviewed the manuscript and provided feedback.

Chapter 3

The author contributed to the design of this study, performed the data collection and data analysis, contributed to the statistical analysis, and prepared the manuscript for publication. A/Professor Donna Geddes, Professor Peter Hartmann and Professor Karen Simmer were involved in the study design. A/Professor Donna Geddes assisted with data collection at the beginning of the study. Ms Anna Hepworth provided statistical support. All co-authors reviewed the manuscript and provided feedback.

Chapter 4

The author contributed to the design of the study, performed the data collection and data analysis, contributed to the statistical analysis and prepared the manuscript for publication. A/Professor Donna, Professor Peter Hartmann and Professor Karen
Simmer were involved in the study design. Ms Anna Hepworth provided statistical support. All co-authors reviewed the manuscript and provided feedback.

Chapter 5
The author contributed to the design of the study, performed the data collection, data analysis, statistical analysis and prepared the manuscript for publication. A/Professor Donna, Professor Peter Hartmann and Professor Karen Simmer were involved in the study design. Ms Anna Hepworth provided statistical consultation and Ms Zoya Gridneva provided assistance with milk sample analysis. All co-authors reviewed the manuscript and provided feedback.

Chapter 6
The author reviewed the literature and wrote the chapter. A/Professor Donna Geddes, Professor Peter Hartmann and Professor Karen Simmer reviewed the chapter and provided feedback.
Table of Contents

Preface .............................................................................................................................................. i
Abstract ........................................................................................................................................ ii
Acknowledgements .................................................................................................................. v
Publications ..................................................................................................................................... vi
Statement of Candidate Contribution .................................................................................. viii
Table of Contents .................................................................................................................... x
List of Figures .......................................................................................................................... 15
List of Tables ............................................................................................................................. 16
Abbreviations and Units ........................................................................................................... xviii

Chapter 1 Gastric Empting and Curding of Breastmilk in Preterm Infants .......... 1
  1.1 Introduction ...................................................................................................................... 1
  1.2 Preterm enteral feeding ............................................................................................... 2
  1.3 Feeding intolerance ..................................................................................................... 3
  1.4 Gastric emptying of breastmilk in preterm infants ................................................... 5
  1.5 Regulation of gastric emptying ................................................................................... 6
    1.5.1 Gastro-duodenal motor activity .......................................................................... 6
    1.5.2 Enteroendocrine regulation ............................................................................... 7
  1.6 Gastric emptying monitoring methods ...................................................................... 8
    1.6.1 Intubation techniques ......................................................................................... 9
      1.6.1.1 Gastric aspiration ...................................................................................... 9
      1.6.1.2 Dilution marker methods .......................................................................... 9
    1.6.2 Indirect techniques ............................................................................................. 11
      1.6.2.1 Paracetamol (acetaminophen) absorption test .......................................... 11
      1.6.2.2 Stable isotope breath test ......................................................................... 12
      1.6.2.3 Gastric electrical impedance ..................................................................... 13
      1.6.2.4 Applied potential tomography .................................................................... 14
      1.6.2.5 Epigastric impedance ............................................................................... 15
    1.6.3 Imaging techniques .............................................................................................. 17
1.6.3.1 Magnetic resonance imaging ............................................................... 17
1.6.3.1 Scintigraphy .......................................................................................... 18
1.6.3.2 Antral cross sectional area (ACSA) ...................................................... 19
1.6.3.4 Direct ultrasound method ..................................................................... 20

1.7 Intrinsic influences on gastric emptying .................................................. 21
1.7.1 Birth gestation ......................................................................................... 21
1.7.2 Postnatal maturation .............................................................................. 23

1.8 Extrinsic influences on gastric emptying ................................................. 25
1.8.1 Feed volume ........................................................................................... 25
1.8.2 Infant positioning ................................................................................... 27
1.8.3 Non-nutritive sucking ............................................................................. 28
1.8.4 Infant acuity ............................................................................................ 29
1.8.5 Medications ............................................................................................. 30

1.9 Nutrient and biochemical influences on gastric emptying .................... 33
1.9.1 Energy Density ....................................................................................... 33
1.9.2 Carbohydrates ....................................................................................... 34
1.9.3 Protein ..................................................................................................... 36
1.9.4 Fat ........................................................................................................... 39
1.9.5 Feed osmolality ...................................................................................... 41
1.9.6 Donor human milk ............................................................................... 43

1.10 Effects of treatments on breastmilk feed composition ......................... 45
1.10.1 Human milk fortifier ............................................................................ 45
1.10.2 Refrigeration and frozen storage .......................................................... 48
1.10.3 Holder pasteurization .......................................................................... 50

1.11 Intragastric Curding ............................................................................... 52
1.11.1 Ultrasound imaging of stomach contents ........................................... 53

1.12 Assessment of gastric emptying and curding in preterm infants .......... 54

1.13 Aims ....................................................................................................... 54

Chapter 2  Validation of Ultrasound Methods to Monitor Gastric Volume

Changes in Preterm Infants ........................................................................... 56
2.1  Abstract .................................................................................................... 56
2.2  Introduction ............................................................................................. 56
2.2.1 Study Aims ......................................................................................... 58
2.3  Methods .................................................................................................. 59
2.3.1 Participants .......................................................................................... 59
Chapter 2

2.3.2 Study Protocol ........................................................................................... 59
2.3.3 Ultrasound Examination............................................................................ 60
2.3.4 Ultrasound Image Analysis ........................................................................ 61

2.4 Echogenicity of Stomach Contents ............................................................ 62

2.5 Gastric Measurements ............................................................................. 63
  2.5.1 Statistical Analysis ..................................................................................... 63
  2.5.2 Intra and Inter-rater reliability .................................................................. 63
  2.5.3 Validation of Stomach Volume Techniques .............................................. 64

2.6 Results .................................................................................................... 65
  2.6.1 Intra-rater reliability of stomach volume measurements......................... 66
  2.6.2 Inter-rater reliability of stomach volume measurements ........................ 68
  2.6.3 Pre-feed Stomach Residuals ...................................................................... 69
  2.6.4 Relationship between Stomach Calculations and Delivered Milk Volume69
  2.6.5 Characterisation of Stomach Echogenicity ............................................... 71

2.7 Discussion ............................................................................................... 72
  2.7.1 Limitations and Implications ..................................................................... 76

2.8 Conclusion ............................................................................................... 76

Chapter 3

Repeatability of Gastric Volume Measurements and Intragastric Content Using Ultrasound in Preterm Infants .............................................. 77

3.1 Abstract................................................................................................... 77
3.2 Introduction ............................................................................................ 78
3.3 Methods.................................................................................................. 81
  3.3.1 Participants ............................................................................................... 81
  3.3.2 Study protocol ........................................................................................... 81
  3.3.3 Ultrasound Examination............................................................................ 82
  3.3.4 Ultrasound Measurement of Stomach Volume ........................................ 83
  3.3.5 Echogenicity of Stomach Contents ........................................................... 83
  3.3.6 Statistics .................................................................................................... 84
    3.3.6.1 Sample size determination .............................................................................84
    3.3.6.2 Statistical analysis ..........................................................................................84

3.4 Results .................................................................................................... 86
  3.4.1 Infant Characteristics ................................................................................ 86
  3.4.2 Repeatability of Post Feed Stomach Volume Measurements ................. 87
  3.4.3 Repeatability of Immediate Post Feed Stomach Volumes (T0) ................. 89
  3.4.4 Repeatability of Residual Stomach Volumes............................................. 90
3.4.5 Influences on Repeatability of Immediate Post Feed Stomach Volumes (T0) ............................................................................................................ 90
3.4.6 Influences on Repeatability of Stomach Volumes .................................... 91
3.4.7 Repeatability and Patterns of Echogenicity of Stomach Ultrasound Images .................................................................................................................. 91
3.4.8 Repeatability and Patterns of Curd Presence, Density and Volume ....... 95
3.5 Discussion ................................................................................................ 96
3.6 Conclusion ............................................................................................. 102

Chapter 4 Influences on breastmilk composition on gastric emptying of preterm infants ................................................................. 103
4.1 Abstract ................................................................................................. 103
4.2 Introduction .......................................................................................... 104
4.3 Methods ................................................................................................ 106
4.3.1 Participants ............................................................................................. 106
4.3.2 Study protocol ........................................................................................ 106
4.3.3 Analysis of feed composition ................................................................. 107
4.3.4 Ultrasound examination and calculation of stomach volume ............. 107
4.3.5 Statistical analysis ................................................................................... 108
4.4 Results ................................................................................................... 110
4.4.1 Infant characteristics .............................................................................. 110
4.4.2 Feed composition .................................................................................... 111
4.4.3 Influences on gastric emptying during feeding ...................................... 113
4.4.4 Influences on postprandial gastric emptying ......................................... 113
4.4.5 Gastric residual volumes ........................................................................ 117
4.5 Discussion .............................................................................................. 118
4.6 Conclusion ............................................................................................. 122

Chapter 5 Gastric emptying and curding of pasteurized donor human milk and mother’s own milk in preterm infants. .............................. 123
5.1 Abstract ................................................................................................. 123
5.2 Introduction ............................................................................................ 124
5.3 Methods ................................................................................................ 125
5.3.1 Participants ............................................................................................. 125
5.3.2 Study protocol ........................................................................................ 126
5.3.3 Ultrasound examination ....................................................................... 127
List of Figures

Figure 1.1: Gastric emptying of solid and liquid meals ..................................................... 5

Figure 1.2: Regulation of gastric emptying via the ileal brake ........................................... 7

Figure 1.3: Postprandial GIP responses at different postnatal ages for preterm infants born 33.5/40. ................................................................................................................... 8

Figure 1.4: Gastric emptying curves for feeds of differing volumes. .............................. 26

Figure 1.5: Gastric emptying of liquid feeds in supine and prone body positions .......... 28

Figure 2.1: Ultrasound technique for scanning of the preterm infant’s stomach .......... 61

Figure 2.2: Echogenic appearance of intragastric breast milk ........................................ 62

Figure 2.3: Proportion of maximum calculated volume for stomach as a) spheroid, b) hemisphere + cylinder, and c) hemisphere + cone, and for d) antral cross sectional area (ACSA) ..................................................................................... 71

Figure 3.1: Gastric emptying curves for preterm infants fed sequential feeds of a) breastmilk fortified with FM 85 human milk fortifier, b) unfortified breastmilk, and c) breastmilk fortified with S-26 human milk fortifier ..................................................................................... 88

Figure 4.1: Gastric emptying curves for preterm infants fed paired feeds of unfortified mother’s own milk (MOM) and MOM fortified with a) S-26 human milk fortifier or b) FM 85 human milk fortifier ..................................................................................... 115

Figure 5.1: Gastric emptying curves for preterm infants fed paired feeds of unfortified and fortified mother’s own milk (MOM) or pasteurized donor human milk (PDHM). FM-85 (Nestlé) human milk fortifier (HMF) was used to fortify feeds ........................................ 134

Figure 5.2: Serial ratings of gastric content echogenicity recorded immediately post feed (T0) and at 30 minute intervals thereafter for 2 hourly and 3 hourly feeds of unfortified and fortified mother’s own milk (MOM) and pasteurized donor human milk (PDHM) ..................................................................................................................... 138
List of Tables

Table 1.1 Features of gastric emptying monitoring methods for infants ....................... 21

Table 1.2 Macronutrient composition and energy densities of preterm, term, and donor breastmilk reported as mean±standard deviation. ................................................................. 43

Table 1.3 Summary of studies examining differences in emptying of unfortified (UF) and fortified (F) breastmilk in preterm infants. ................................................................. 46

Table 2.1 Intra-rater intra-class correlation coefficients (ICC) for the agreement for repeated image measurements for antral cross sectional area (ACSA) and direct stomach raw measurements and calculations presented as intraclass correlation (95% confidence interval) ........................................................................................................ 66

Table 2.2 Intra-rater mean differences, standard deviations of the differences and limits of agreement for antral cross sectional area (ACSA) and direct stomach raw measurements and calculations of area and volume ................................................................. 67

Table 2.3 Inter-rater mean differences, standard deviations of the differences and limits of agreement for antral cross sectional area (ACSA) and direct stomach measurements. Limits of agreement are mean±(1.96* sd) for 95% certainty .................. 68

Table 2.4 Inter-rater intra-class correlation coefficients (ICC) for the agreement for images measurements for antral cross sectional area (ACSA) and direct stomach raw measurements and calculations presented as intra-class correlations (95% confidence interval) .......................................................................................................................... 69

Table 2.5 Echogenic appearance of breast milk and density and volume of curd during an intra-gastric tube feed ............................................................................................... 72

Table 2.6 Milk type and echogenicity immediately following feed delivery. Echogenicity is determined relative to the spleen, with ‘mixed’ and ‘snow storm’ ratings having a mixture of brighter and darker sections ................................................................. 72

Table 3.1 Biochemical composition and energy content values of S-26 (Wyeth) and FM 85 (Nestlé) human milk fortifiers (HMF) calculated at the prescribed dose for 1L of breastmilk ................................................................. 82

Table 3.2 Infant and feed characteristics for 2 hourly and 3 hourly sequential feed pairs: milk type, human milk fortifier (HMF) type, feed volume in millilitres (mL) and millilitres per kilogram (mL/kg), feed duration, infant corrected gestational age, postnatal age and weight ................................................................................................. 86

Table 3.3 Agreement between ratings of echogenicity and curd at all time points for sequential feeds of unfortified breastmilk and breastmilk fortified with human milk fortifier (HMF) as assessed by Kappa and 95% Confidence Intervals (95% CI) ............ 93
Table 3.4 Agreement between ratings of echogenicity and curd at each matched time point for sequential feeds of unfortified and fortified breastmilk as assessed by Kappa and 95% Confidence Intervals (95% CI). .................................................................94

Table 4.1 Feed tallies for mother’s own milk (MOM) and fortified MOM feed pairs by feed frequency and human milk fortifier (HMF) type, and infant and feed characteristics reported as mean±SD (range). ........................................................................111

Table 4.2 Biochemical composition (g/L) and energy content (cal/30 mL) values of S-26 and FM 85 human milk fortifiers (HMF) at the prescribed dose for 1L of milk, and of unfortified mother’s own milk (MOM) and MOM fortified with S-26 HMF and FM 85 HMF reported as mean±SD (range). ..............................................................................112

Table 4.3 Significance (p-values) of selected predictors for measured stomach volumes (volume) and calculated proportions of delivered feed volume (% feed) immediately post feed (T0) and for subsequent serial post feed time points. .................................116

Table 4.4 Gastric residual volumes for mother’s own milk (MOM) feeds reported for all feeds, unfortified MOM, MOM fortified with S-26 human milk fortifier (HMF) and MOM fortified with FM 85 HMF. .................................................................................. 118

Table 5.1 Infant and feed characteristics for 2 hourly and 3 hourly unfortified and fortified feed pairs of mother’s own milk (MOM) and pasteurized donor human milk (PDHM) where one feed was fortified with FM-85 (Nestlé) human milk fortifier. Data are presented as mean±standard deviation, (range) ................................................... 131

Table 5.2 Biochemical concentration (g/L) and energy concentration (cal/30 mL) values (mean±standard deviation, range) of unfortified mother’s own milk (MOM) and pasteurized donor human milk (PDHM), FM-85 (Nestlé) human milk fortifier (HMF), and fortified MOM and PDHM ...................................................................................... 132

Table 5.3 Significance (p-values) of selected predictors for measured stomach volumes (volume) and calculated proportions of delivered feed volume (% feed) immediately post feed and for subsequent serial postprandial time points after feeds of mother’s own milk (MOM) and pasteurized donor human milk (PDHM) ........................................................................................................ 135

Table 5.4 Gastric residuals reported for all feed types. Counts of empty stomachs (stomach volume=0 mL), residual volumes >0 mL, residual volumes >3 mL (Volume >3 mL) and volumes >30% of delivered feed volume (Volume >30% feed) for 2 hourly feeds at 90 min post feed and 3 hourly feeds at 150 min post feed ........................................ 137

Table 5.5 Serial counts and proportions (%) of gastric curd volume and density ratings after 2 hourly and 3 hourly feeds of mother’s own milk (MOM) and pasteurized donor human milk (PDHM), both unfortified and fortified with FM-85 (Nestlé) human milk fortifier (HMF) .............................................................................................................. 139
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>/40</td>
<td>weeks of gestation</td>
</tr>
<tr>
<td>$^{13}$COABT</td>
<td>13C octanoic breath test</td>
</tr>
<tr>
<td>ACSA</td>
<td>Antral cross sectional area</td>
</tr>
<tr>
<td>APT</td>
<td>Applied potential tomography</td>
</tr>
<tr>
<td>BSSL</td>
<td>Bile salt stimulated lipase</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CGA</td>
<td>Corrected gestational age</td>
</tr>
<tr>
<td>CPAP</td>
<td>Continuous positive airway pressure</td>
</tr>
<tr>
<td>cpm</td>
<td>Cycles per minute</td>
</tr>
<tr>
<td>EI</td>
<td>Electrical impedance</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GIP</td>
<td>Gastric inhibitory polypeptide</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide 1</td>
</tr>
<tr>
<td>GORD</td>
<td>Gastro-oesophageal reflux disease</td>
</tr>
<tr>
<td>HMF</td>
<td>Human milk fortifier</td>
</tr>
<tr>
<td>LCT</td>
<td>Long chain triglycerides</td>
</tr>
<tr>
<td>MCT</td>
<td>Medium chain triglycerides</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mL/kg</td>
<td>Millilitres per kilogram</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>MOM</td>
<td>Mothers own milk</td>
</tr>
<tr>
<td>NEC</td>
<td>Necrotising enterocolitis</td>
</tr>
<tr>
<td>NNS</td>
<td>Non-nutritive sucking</td>
</tr>
<tr>
<td>PDHM</td>
<td>Pasteurized donor human milk</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide tyrosine tyrosine</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>Gastric half emptying time</td>
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Chapter 1 Gastric Empting and Curding of Breastmilk in Preterm Infants

1.1 Introduction

Major advances in neonatal intensive care over the last 3 decades have resulted in reduced mortality rates and improved outcomes for infants born preterm (<37 weeks gestation; 37/40), particularly for those born very preterm (28 to <32/40) and extremely preterm (<28/40 weeks) (Allen et al., 2011, Richardson et al., 1998, World Health Organization, 1977). Significant improvements are largely attributed to more proactive and advanced cardiovascular and respiratory management due to the groundbreaking and prolific research performed in these areas (Allen et al., 2011). However preterm birth poses a nutritional emergency, and is now a prominent focus of the neonatologist with the pertinent problem of postnatal growth restriction associated with increased morbidity including poor physiological, neurodevelopmental and metabolic outcomes (Corpeleijn et al., 2011). Newborn preterm infants quickly develop protein and energy deficits, and it is an ongoing challenge to compensate for this through enteral feeding of relatively small feed volumes (Corpeleijn et al., 2011, Embleton et al., 2001). Fortification of breastmilk therefore is recommended for very low birth weight infants (VLBW, birth weight <1500 g) yet presents another dilemma to deliver the delicate balance of required protein, energy and micronutrients (American Academy of Pediatrics, 2012, Quigley and McGuire, 2014, Eidelman, 2012). The optimal composition of human milk fortifier (HMF) is yet to be determined (Arslanoglu et al., 2010), and neonatologists have no means of readily assessing the feeding tolerance of new HMF products.

Fortified breastmilk is considered optimal for VLBW infants due to the advantages of breastmilk that include stimulation of gut maturation through insulin-like growth factors 1 and 2 and endothelial growth factor (Lonnerdal, 2003), a faster progression to full enteral feeds, enhanced feeding tolerance (Sisk et al., 2008), and lower rates of mortality and morbidities including sepsis, necrotizing enterocolitis.
Chapter 1

(NEC) and severe retinopathy of prematurity (American Academy of Pediatrics, 2012, Boyd et al., 2007, Corpeleijn et al., 2012). In the longer term, breastmilk-fed preterm infants have fewer illness-related hospital readmissions in the year following discharge, as well as improved neurodevelopmental outcomes at eight years of age and in adolescence (American Academy of Pediatrics, 2012). Another advantage is that breastmilk forms softer curds and empties from the stomach faster than formula (Cavell, 1981, Ewer et al., 1994) and so is less likely to result in large gastric residual volumes that are associated with NEC and feeding intolerance. Gastric residual volumes are routinely measured through aspiration of the intragastric tube, yet evidence indicates this method is not reliable (Cavell, 1981, Metheny et al., 2005).

Unfortunately not all mothers are able to produce the volume of breastmilk required to meet their preterm infants’ needs and approximately 72% of infants require supplementation with an alternative source of nutrition (Carroll and Herrmann, 2013). In this situation donor milk is considered the preferred alternative (American Academy of Pediatrics, 2012, World Health Organization and UNICEF, 2003) although it is typically subjected to frozen storage and heat pasteurization, both of which alter breastmilk composition and bioactivity (American Academy of Pediatrics, 2012, Garcia-Lara et al., 2013). Currently the impact of donor milk is unknown with respect to curding and gastric emptying.

While feeding intolerance, associated with delayed gastric emptying, remains a common problem for preterm infants, reliable monitoring methods and parameters for both normal and delayed gastric emptying have not been established. Thus clinical access to accurate and non-invasive methods for tracking gastric emptying, measurement of residual stomach volumes and evaluation of feeding tolerance in response to different types of nutrition or management is imperative in the quest to improve neonatal nutrition.

1.2 Preterm enteral feeding

The healthy fetus receives a continuous nutrient supply via the umbilical cord yet nearly always has a fluid-filled stomach (Sase et al., 2002). Birth at <34/40 poses a
nutritional emergency as the infant has high nutritional needs but lacks the capacity to suck full oral feeds. While the newborn term infant ingests small volumes of colostrum with subsequent increasing volumes during the transition to breastmilk (Casey et al., 1986, Saint et al., 1984), for the preterm infant born <34/40, the initiation and progression of enteral feeding is typically delayed due to immature coordination of sucking, swallowing and breathing, feeding intolerance and co-morbidities (Neiva et al., 2014, Morgan et al., 2013a, Morgan et al., 2013b, Jadcherla et al., 2010).

Initially parenteral nutrition is given while the infant fasts or receives minimal enteral feeds via an intragastric tube (Morgan et al., 2013a). Interestingly, fasting disrupts the already aberrant gut colonization of the preterm infant and gut atrophy may result from the absence of feeding-stimulated local growth factor peptides (Jacobi and Odle, 2012), potentially increasing the infant’s risk of feeding intolerance and NEC (Neu and Walker, 2011, Di Mauro et al., 2013, Indrio et al., 2008). Parenteral fluids are gradually replaced by intragastric tube feeds whereby the milk bypasses the mouth and oesophagus, potentially reducing exposure to lingual digestive enzymes such as amylase and lipase (Hamosh et al., 1981, Murray et al., 1986). Enteral feeding is often slowed or interrupted when infants show signs of difficulty in tolerating feeds.

1.3 Feeding intolerance

Feeding intolerance is a common problem in preterm infants, characterized by signs of delayed gastric emptying such as large gastric aspirates, abdominal distension and/or vomiting that may herald the onset of NEC (Moore and Wilson, 2011). Disruption of infant feeding in response to feeding intolerance contributes to suboptimal nutrition and faltering postnatal growth (Fanaro, 2013, Miller et al., 2014). The aetiology of feeding intolerance is not well understood although immature gastroduodenal motility and an altered gut microbiota may contribute (Riezzo et al., 2000, Riezzo et al., 2009, Ittmann et al., 1992, Di Mauro et al., 2013, Indrio et al., 2008). In the absence of quantitative measures of gastrointestinal function, identification of feeding intolerance is based on gross clinical signs such as pre-feed gastric residual volumes.
A gastric residual that is >30% - 50% of the previous feed volume is considered to be clinically significant although normal standards for gastric residuals have not been established (Lucchini et al., 2011, Moore and Wilson, 2011). Evidence suggests that gastric residuals >2 mL/kg are not associated with delayed attainment of full enteral feeding and therefore residuals are probably not useful in guiding the advancement of preterm feeding (Shulman et al., 2011, Moody et al., 2000). However, gastric residuals >30% to 50% of the delivered feed volume remains an important clinical sign because of the association with NEC (Cobb et al., 2004, Lin and Stoll, 2006, Bertino, 2009 #479). When compared to healthy infants, those with NEC had a higher incidence of gastric residuals ≥ 0.5 mL (1 in 8 feeds vs. 1 in 16 feeds, p<0.01) and higher average residual feed proportions (40% vs. 14%, p<0.001) prior to the onset of NEC (Cobb et al., 2004). As the observed ranges of residual volumes likely overlap between healthy and pre-NEC infants, residual volumes alone are not a reliable predictor of NEC and must be considered in conjunction with other antecedent signs (Jadcherla and Kliegman, 2002).

Abdominal distension is a common sign of feeding intolerance that is characterized by stretching of skin in the epigastric region. It is thought to reflect delayed gastric emptying and may indicate the onset of NEC when accompanied by abdominal wall erythema, reduced bowel sounds and/or systemic signs as well as other signs of feeding intolerance (Lucchini et al., 2011). Serial abdominal girth measurements are made when NEC is suspected, with a girth increase of >2cm between feeds associated with a residual of >23% of the feed volume. (Lucchini et al., 2011, Malhotra et al., 1992). Girth measurements provide confirmation of a clinical sign rather than a sensitive diagnostic measure, and reliability has not been established. At best girth measurements provide an adjunct to initial clinical investigations of suspected NEC.

Delayed gastric emptying can persist after the resolution of obvious signs of feeding intolerance. The time taken for half of the delivered feed volume to empty from the stomach, or ‘gastric half emptying time’ (T½) has been compared between healthy preterm infants and those with feeding intolerance both during and after its
resolution using serial antral cross-sectional area (ACSA) measurements. Compared to healthy infants, the $T\frac{1}{2}$ of those recovered from feeding intolerance was significantly longer, and within the latter group, $T\frac{1}{2}$ was significantly shorter for breastmilk fed infants compared to those fed formula (Carlos et al., 1997). Milk type and composition are therefore likely to be important modulators of feeding intolerance.

1.4 Gastric emptying of breastmilk in preterm infants

Gastric emptying is the process by which a feed leaves the stomach and passes into the duodenum. It is evident in the human fetus from the beginning of the second trimester of pregnancy with swallowed amniotic fluid and pulmonary secretions emptied from the stomach (Sase et al., 2005b). Gastric contractions and the volume of fluid emptied from the stomach progressively increase from 24/40, suggesting an effect of maturation on fetal gastric emptying (Sase et al., 2000).

Liquid feeds have a biphasic pattern of emptying with an initial rapid emptying phase that has been observed in infants, and contrasts with solid meal emptying that is characterized by a lag phase prior to commencement of emptying (Siegel et al., 1988) (Figure 1.1). Gastric emptying is thought to be delayed in infants born <34/40 (Neu, 2007).

![Figure 1.1: Gastric emptying of solid and liquid meals. Modified from (Urbain & Charkes, 1995).](image-url)
1.5 Regulation of gastric emptying

1.5.1 Gastro-duodenal motor activity

Gastro-duodenal motor activity regulates gastric emptying by moving nutrients distally from the stomach to the intestine and matures throughout gestation and early infancy (Berseth, 1996, Liang et al., 1998). Immature motor patterns may contribute to the slower emptying observed in very preterm infants (American Academy of Pediatrics, 2012, Berseth, 1996, Horowitz and Dent, 1991). Several electrogastrography (EGG) studies have been completed in preterm infants at different gestational ages, providing a measure of myoelectrical signals that control gastric motor activity. Normal slow wave activity is considered to be 2–4 cycles per minute (cpm), with bradygastria <2 cpm and tachygastria 4–9 cpm (Parkman et al., 2003). Proportions of normal slow waves are similar between infants born >32/40 and term infants regardless of feed type and volume (Chen et al., 1997, Koch et al., 1993, Precioso et al., 2002, Riezzo et al., 2000, Riezzo et al., 2009) while there is limited but conflicting evidence of gastric motor activity patterns in very preterm infants. Two studies report similar findings between infants born 28-32/40 (n=7) (Koch et al., 1993), 28-31/40 (n=15) (Precioso et al., 2002) and >32/40 including those born at term while Riezzo et al. reported a higher proportion of tachygastria (59.3%) in infants born 28-32/40 (n=12) (Riezzo et al., 2000). Riezzo did not report infant postnatal age, while postnatal ages for the other studies were 9 to 51 days (Koch et al., 1993) and 2 weeks of age (Precioso et al., 2002). In the latter two studies, most of the infants born <32/40 had corrected ages >32/40 and therefore the effects of postnatal maturation and exposure to enteral feeding may have confounded the results.

When compared to antral and duodenal motor activity in term infants, those born at 29/40 (range 25–35/40) have mature antral activity but immature duodenal activity resulting in poor antral-duodenal motor coordination (Ittmann et al., 1992). However mature responses develop with increasing gestational age and are accelerated with exposure to enteral feeds (al Tawil and Berseth, 1996, Ittmann et al., 1992).
1.5.2 Enteroendocrine regulation

The rate of emptying from the stomach is regulated by the ileal brake whereby the rate of gastric transit to the intestine in adjusted in response to the nutrient load of the ingested meal to achieve a constant rate of delivery and optimize digestion and absorption of nutrients (Hellstrom et al., 2006, Shin et al., 2013). Enteroendocrine cells, typically concentrated in the small intestine, detect the osmotic load and various nutrient end products of digestion and secrete enterogastrone hormones such as gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), and peptide tyrosine tyrosine (PYY) that are released into the blood stream, diffused as local messengers, or transmitted to the enteric nervous system (Figure 1.2).

Figure 1.2: Regulation of gastric emptying via the ileal brake

Enterogastrone trigger the ileal brake resulting in relaxation of the gastric fundus, suppression of antral contractions, increased pyloric sphincter pressure and slowed duodenal motor activity that inhibits the forward movement of gastric and intestinal contents (Gribble, 2012, Shin et al., 2013, Van Citters and Lin, 2006). While each macronutrient can stimulate enterogastrone release, the timing and size of the effects of release differs between hormones (Schirra and Goke, 2005, Shin et al., 2013, Marathe et al., 2013). The detection of hyperosmolar gastric contents by duodenal osmoreceptors results in delayed gastric emptying and increased gastric secretions, in effect reducing the osmotic load of the gastric contents (Pearson et al., 2013).

Gastroenterone secretion differs between adults and infants, with higher fasting serum and postprandial levels of GLP-1 observed during the neonatal period (Berseth et al., 1992, Padidela et al., 2009). Compared to term infants, preterm infants
have significantly higher fasting GLP-1, GIP, PYY and CCK levels that persist across the first 10 postnatal weeks for GLP-1, GIP and PYY (Kawamata et al., 2014, Padidela et al., 2009, Siahanidou et al., 2007). Fasting serum PYY and GLP-1 levels are lower with increasing gestational age (Berseth et al., 1992, Kawamata et al., 2014), while the expected postprandial rise in GIP is absent at 6 and 13 days but present at 24 days after birth (Figure 1.3) (Lucas et al., 1980). It is not known whether the higher serum levels and altered GIP response influence the response of the ileal brake and subsequent gastric emptying in preterm infants. Accurate serial measures of gastric emptying are required to detect differences in emptying that results from feeds of varying compositions.

Figure 1.3: Postprandial GIP responses at different postnatal ages for preterm infants born 33.5/40. (Modified from Lucas, Bloom & Aynsley-Green, 1980).

1.6 Gastric emptying monitoring methods

Several methods of monitoring gastric emptying have been trialed in the preterm population. However, these methods are restricted by imprecise or limited information, or by factors that make the method unsuitable for use with fragile preterm infants (Table 1.1). Further, variation and repeatability of gastric emptying measures have not been adequately established in the preterm population.

Methods of assessing gastric emptying can be categorized according to the techniques used, such as intubation techniques, indirect and imaging techniques.
Scintigraphy is generally considered the gold standard (Lin, 2005), however intubation techniques that involve serial aspiration and measurement of gastric contents, are the most clinically practical method available for infants.

1.6.1 Intubation techniques

1.6.1.1 Gastric aspiration

The gastric aspiration method provides an indication of gastric emptying over time. Following delivery of a liquid feed, gastric contents are aspirated, measured and returned at set intervals via an intragastric tube. Residual volumes are reported as proportions of the delivered feed volume, and the T½ is estimated from the data (Husband and Husband, 1969).

Refractometry is reported to increase accuracy of the aspiration method by accounting for gastric secretions when determining the residual feed volume. Refractometry, or determination of the total soluble solids in solution or Brix value of the test meal and subsequent aspirates is used in mathematical equations to differentiate the retained feed volume from gastric secretions (Chang et al., 2004). A close correlation of serial Brix values with increasingly dilute test formula has been confirmed in a laboratory study (Chang et al., 2005). Testing of the mathematical equation in adults following milk feeds indicated that on average 85% (standard deviation:±12%) of gastric aspirates were retained feed, with the remainder assumed to be gastric secretions (Chang et al., 2007), however as intragastric curd was not accounted for, the results only apply to the aspirated liquid portion of the feed. Further, as a comparison of results from gastric aspiration alone and in conjunction with refractometry demonstrated a high level of agreement it is unlikely that refractometry is of value in the clinical setting (Moreira and McQuiggan, 2009).

1.6.1.2 Dilution marker methods

The dye dilution method provides a proxy measure of gastric emptying. The stomach is washed out via an intragastric tube to remove gastric secretions prior to delivery of a test meal containing a non-absorbable dye marker such as phenosulfonphthalein (phenol red) or polyethylene glycol (PEG). Marker dye concentrations of serial gastric aspirate samples are measured and used to estimate

The method has been adapted to minimize analytical errors whereby the marker is withheld from the test meal, and an increasing prescribed dose of dye marker is delivered prior to each sampling episode (Hurwitz, 1981). Photometrical analysis of samples determines dye marker concentrations, providing proxy measures of gastric volume. As the first measurement of dye marker concentration is made after test feed delivery, this method does not take into account emptying that occurs during meal delivery.

Dilution marker methods have been used in preterm infants for breastmilk or formula test feeds (Siegel et al., 1985, Siegel et al., 1982, Siegel et al., 1984, Yu, 1975, Cavell, 1979, Cavell, 1981, Cavell, 1982). However, milk proteins have been shown to impair concentration analyses and so use of a clear liquid test meal is recommended to ensure accurate photometric measurement of the marker substance (Ruhl et al., 1995). The delivery of adequate nutrition is a continuing challenge for very preterm infants so replacement of milk with a clear liquid test meal may not be clinically appropriate (Corpeleijn et al., 2011). Further, gastric emptying is influenced by feed composition, so gastric emptying data obtained from a clear liquid test meal are unlikely to reflect that of milk feeds. Lastly, PEG significantly delays gastric emptying in adults and so can confound results of the dye dilution method (Coremans et al., 2005).

Advantages of the intubation techniques are the ability to be performed at the bedside, and the relatively low cost of the procedure. However there are several concerns regarding the suitability and accuracy of this method in the preterm population. Intubation methods are invasive, requiring washing of the gastric lumen and aspiration of stomach contents and that may interfere with the protective acidic environment and microbiome of the stomach (Milisavljevic et al., 2013, Kelly and Newell, 1994). Further, as feed volume likely impacts the rate of gastric emptying (Kwiatek et al., 2009, Schmitz et al., 2012) repeated aspiration and replacement of stomach contents may affect gastric motility and emptying and therefore confound results. Correct placement of the intragastric tube is clearly critical to enable aspiration of all fluid from the stomach, however reported rates of correct tube placement are low at 47.5 to 74% in neonates (de Boer et al., 2009, Freeman et al., 2012). Moreover,
there is evidence that the influences of intragastric tube size, syringe size and body positioning further contribute to the unreliability of gastric aspirates as measures of stomach content volume (Metheny et al., 2005). For preterm infants the intragastric tube size is typically 5 or 6 French gauge, with an external diameter of 1.7 – 2 mm. As gastric curd may not be easily aspirated through the narrow bore of an intragastric tube, it is feasible that aspirate volumes reflect only the fluid portion of a feed, thus underestimating the total volume of stomach contents. Finally, as the intubation techniques have not been validated in the preterm population, they are not suitable for assessing gastric emptying in preterm infants.

1.6.2 Indirect techniques

1.6.2.1 Paracetamol (acetaminophen) absorption test

The paracetamol absorption test provides a proxy marker for gastric emptying of liquid meals. After a period of fasting a test meal is ingested together with a specific dose of paracetamol, and postprandial serial blood assays are collected. While gastric absorption of paracetamol is negligible, it is completely absorbed in the small intestine so serial blood assay levels reflect the increasing proportions of test meal that pass into the small intestine over time (Willems et al., 2001, Heading et al., 1973). Gastric emptying curves and T½ are reported for this method.

The paracetamol absorption test generally correlates well with scintigraphic measures of liquid meal emptying, is inexpensive and simple to perform in adults (Willems et al., 2001, Glerup et al., 2007, Naslund et al., 2000). However, accuracy may be confounded by inter-individual variations in the metabolism of paracetamol, and by drug interactions (Kim et al., 2000). Paracetamol can be administered to infants from 30/40 but its absorption is delayed and clearance times decrease with maturation suggesting the test may not be a reliable measure of gastric emptying in preterm infants (Anderson et al., 2002, Cuzzolin et al., 2013). Use and validation of the paracetamol absorption test have not been reported in the neonatal population. Further there is limited safety data for paracetamol use in this population (Cuzzolin et al., 2013) and the requirements for fasting and serial blood sampling are to be avoided in the preterm population where interruption of feeding is not advisable and anaemia is common (Corpeleijn et al., 2011, Strauss, 2010).
1.6.2.2 Stable isotope breath test

The stable isotope breath test provides an indirect measure of gastric emptying whereby a test meal labeled with a stable isotope is given and elimination of the isotope is measured through serial breath sampling. The stable isotope, most commonly $^{13}\text{C}$, is cleaved from a substrate such as octanoic acid (Ghoos et al., 1993), acetate (Barbosa et al., 2005) or sodium octanoate (van Wijk et al., 2007) during digestion in the duodenum, transported to the liver where it is oxidized to produce $^{13}\text{CO}_2$ and eliminated via the lungs (Ghoos et al., 1993, Moreira and McQuiggan, 2009). Breath samples are collected via a mask and the expired $^{13}\text{C}$ fraction of the total $^{13}\text{C}$ substrate is analysed using mass or infrared spectrometry (Ghoos et al., 1993, Hauser et al., 2006). The reported cumulative proportion of recovered $^{13}\text{C}$ dose is inversely related to the proportion of test meal remaining in the stomach, and the reported breath test curve illustrates the proportion of $^{13}\text{C}$ dose excreted per hour. While the breath test curve itself is not physiologically meaningful, nonlinear regression analysis allows for calculation of $T_{1/2}$, and estimation of the lag time following ingestion of a solid meal. The gastric emptying coefficient, an overall index of gastric emptying, is also calculated from the data as a natural logarithm (Maes et al., 1994).

Stable isotope breath tests have been validated against scintigraphy in adults (Ghoos et al., 1993) and older infants with gastro-oesophageal reflux disease (Barbosa et al., 2005), and trialed in three small studies of preterm infants (Pozler et al., 2003, van Wijk et al., 2007, Veereman-Wauters et al., 1996). The $^{13}\text{C}$ octanoic acid breath test ($^{13}\text{COABT}$) was adapted for use in preterm infants ($n=11$) by standardizing the test meal and sampling technique, and adjusting calculations to reflect the expected $\text{CO}_2$ production of infants. The addition of PEG was required to make the $^{13}\text{C}$ substrate adequately soluble for addition to the infant formula test meal, and so may have confounded results through its inhibition of gastric emptying (Coremans et al., 2005).

Van Wijk et al. used combined oesophageal impedance manometry in conjunction with the $^{13}\text{C}$ Na-octanoate breath test to examine the effects of body positioning on gastro-oesophageal reflux and gastric emptying in 10 preterm infants (van Wijk et al., 2007). The $T_{1/2}$ calculated from breath test results indicated significant differences between left lateral and right lateral body positions. Minimal details of the experience of infant breath test sampling or of analysis were reported. Pozler et al.
evaluated the use of the $^{13}$COABT in 16 preterm infants and noted several anomalous findings that were omitted from the analyses of repeatability of $T_{1/2}$ and intra-individual variation. These included measured proportions of $^{13}$C dose that did not increase or decrease as expected, postprandial values that were lower than baseline measures, and curves did not return to baseline values (Pozler et al., 2003). These unusual findings call into question the reliability of stable isotope methods in the preterm population.

Absorption, metabolism and excretion of $^{13}$CO$_2$ are dependent on hepatic, pulmonary and duodenal function (Keller et al., 2009). It is possible that respiratory disease and/or the immature biotransformative function of the infant’s liver impact on $^{13}$CO$_2$ excretion and thus the validity of the breath test in the preterm population. Further, the use of a mask or nasopharyngeal tube for breath sampling excludes infants requiring respiratory support by means of supplemental oxygen, continuous positive airway pressure (CPAP) or mechanical ventilation (Pozler et al., 2003, van Wijk et al., 2007). The requirement for fasting prior to the breath test makes this method unsuitable for infants with residual feed volumes present such as those receiving continuous or frequent intermittent feeds. Also, the equipment and expertise required for the procedure and data analysis are not readily available in the neonatal nursery setting.

### 1.6.2.3 Gastric electrical impedance

Gastric electrical impedance methods include applied potential tomography (APT) and epigastric impedance (EI) whereby measures of gastric electrical impedance or resistivity recorded over the postprandial period reflect gastric emptying. Electrodes are placed on the subject’s trunk, a low frequency electrical alternating current is applied, and a resistivity trace is produced from measurements of current from the body surface (Brown et al., 1985). When the test feed has a higher conductivity than the stomach wall, feed delivery results in an increase in resistivity that subsequently decreases as the stomach empties (Brown, 2003). The point at which the resistivity value is half that of the immediate post feed value reflects the gastric half emptying time (Nour et al., 1995). As resistivity varies in response to changes in pH, pharmacological suppression of gastric acid secretion by H$_2$ receptor antagonists such
as ranitidine or cimetidine may be used for gastric electrical impedance studies (Brown, 2003, Mangnall et al., 1988, Mangnall et al., 1987).

1.6.2.4 Applied potential tomography

Applied potential tomography (APT) involves the application of a current through sixteen electrodes that are placed in a circular array around the trunk at the level of the gastric fundus or body (Mangnall et al., 1987) or antrum (Brown et al., 1985). APT has been trialed in a study of preterm infants (corrected age ~34/40, n=53) and young infants <3 months of age (n=29) using infant formula, breastmilk or Dioralyte feeds (Nour et al., 1995). As these feeds do not adequately conduct an electrical current, the addition of sodium chloride was required to increase conductivity and provide a satisfactory trace.

Reported comparisons of APT and scintigraphic measures of gastric emptying of liquid meals are not conclusive. Mangall et al. (Mangnall et al., 1988, Mangnall et al., 1987) reported a coefficient of correlation of 0.80 in adults (n=6), while Podczeck et al. compared graphical representations of emptying for each method (n=10) and concluded that the two methods do not give equivalent results, with the longer emptying time indicated by scintigraphy potentially confounded by gastric secretions and radioactive scatter from the test meal in adjacent intestine (Podczeck et al., 2007). Interestingly, gastric acid suppression medication was used only in the study that reported a high correlation between APT and scintigraphy.

Nour et al. compared ATP and aspirated gastric residual volumes in preterm and term infants (n=47). For each feed, ATP and residual volumes were measured and calculated residual feed proportions compared, Differences of <25% between the paired values were considered to be in agreement (Nour et al., 1995). Results were similar with 85% agreement for paired calculations, however statistical analysis was not reported and the criteria for agreement were broad. Specifically, while emptying of >75% of the feed volume was reported for 15/20 and 16/20 breastmilk or formula feeds, and 25/27 and 26/27 of Dioralyte feeds by APT and aspiration respectively, 23% (11/47) had discrepancies of 20 – 100% between paired calculations based on APT and gastric aspirate measurements.
1.6.2.5 Epigastric impedance

Epigastric impedance (EI) uses a single-channel electrical configuration to apply a current in a cross-sectional plane over the gastric area, with continuous readings of electrical flow used to produce an impedance trace that reflects both gastric emptying and motility (Lange et al., 1997, Huerta-Franco et al., 2012). Two or four electrodes are applied anteriorly and posteriorly over the area of the stomach to apply and read electrical flow.

EI was used to study gastric emptying in term (n=26) and preterm (n=4) using water 5 mL/kg as a test meal as the conductivity of breastmilk and formula were considered too low to produce adequate impedance traces (Lange et al., 1997). Repeatability was studied on two consecutive days (n=12) with analysis of complete data (n=9) showing a $T\frac{1}{2}$ coefficient of variation of 17%. Only 74% of all study data were suitable for analysis due to 16% movement related invalid measurements and 10% anomalous resistivity measurements (Lange et al., 1997).

Results of validation studies comparing EI and scintigraphic measures of liquid gastric emptying are inconsistent. In adult studies, the coefficients of correlation were 0.86 (n=6) for emptying curve slopes (Sutton et al., 1985), and 0.27 (n=8) for $T\frac{1}{2}$ (Mangnall et al., 1988) despite the latter study using pharmacological gastric acid suppression that is thought to improve EI data acquisition.

Use of EI was evaluated in 45 children (7 months – 17 years of age) comparing incremental delivery of a liquid test meal of orange squash with stepwise changes in impedance, as the authors felt it unethical to validate the method against scintigraphy or dye dilution techniques in children (Smith et al., 1993). The increase in ingested volume correlated highly ($r >0.95$) with the increase in impedance. Orange squash was given so as to prevent the stimulation of gastric acid secretion that is seen with milk feeds. To prevent movement artifact, children younger than 4 years of age were pharmacologically sedated; this practice would not be appropriate for preterm infants in whom apnoea of prematurity is a common problem (Zhao et al., 2011). In a study of formula fed infants with gastric motility disease at ages 1–6 months (n=7) a correlation coefficient of 0.86 was reported (Savino et al., 2004). The authors stated that spontaneous infant movements caused spurious recordings, and treatment of this data with regard to data analysis was not described.
Gastric electrical impedance methods provide safe, non-invasive and low cost monitoring of gastric emptying with rapid data collection that can be performed at the bedside (Brown, 2003, Huerta-Franco et al., 2012). Direct comparisons of gastric emptying measures of APT and EI with scintigraphy indicate that when pharmacological acid suppression is used, APT measures are more repeatable and reliable than that of EI (Mangnall et al., 1988). However, with few and conflicting validation studies, the repeatability and reliability of both APT and EI as methods of measuring gastric emptying in adults and children are yet to be established.

Gastric electrical impedance methods are sensitive to input from changes in resistivity from neighbouring structures such as the oesophagus, duodenum and large intestine and movement as well as acidity and meal resistivity (Smith et al., 1993, Lange et al., 1997, Huerta-Franco et al., 2012).

Children <4 years have been chemically sedated to exclude movement artifact, while another study reported no effect of infant body movements and crying on resistivity readings (Nour et al., 1995). Input from adjacent gastrointestinal organs has not been reported in paediatric studies as a complicating factor in data collection. The effects of body movement and external input on resistivity readings requires further evaluation.

The use of a test meal other than milk is not acceptable in the neonatal setting, as it disrupts the essential supply of enteral nutrition. However milk is considered to have inadequate reactivity to produce a gastric emptying curve, and the addition of a conductive solution such as sodium chloride may be required (Lange et al., 1997, Nour et al., 1995). As gastric electrical impedance methods are restricted to either particulate solid or liquid (Mangnall et al., 1987) but not mixed meals, it is unlikely these methods would accurately reflect emptying of milk feed that separate into liquid (whey) and semi-solid (casein curd) phases within the stomach. The selection of an optimal test meal is problematic.

Interestingly while gastric acid suppression is often considered necessary in adult electrical impedance studies, Nour et al. did not consider it necessary for infants undergoing APT on the basis of already reduced acid production (Nour et al., 1995). This is not congruent with the published literature which states that preterm infants
are able to maintain a stomach pH <4.0 from birth (Kelly et al., 1993), and postprandial re-acidification occurs more rapidly than previously thought with the stomach pH <4.0 for more than half of the inter-feed interval (Omari and Davidson, 2003). Suppression of gastric acid production in the preterm infant would interrupt physiological processes such as the digestion of proteins and bacteria and so it is to be avoided (Henderson et al., 2001, Kelly et al., 1993, Zhu et al., 2006).

Gastric electrical impedance methods of monitoring gastric emptying in preterm infants are potentially appealing as they are non-invasive and may be adapted for use in the clinical setting. However, the unsuitability of milk as a test feed and potential interference from infant movement indicate that this method is not suitable.

1.6.3 Imaging techniques

1.6.3.1 Magnetic resonance imaging

Evidence from a number of adult studies suggests that magnetic resonance imaging (MRI) may offer a non-invasive yet detailed method to simultaneously quantify gastric emptying and motility, providing details of stomach volume, distribution of stomach contents and gastric distension in response to meal ingestion (de Zwart and de Roos, 2010, Schwizer et al., 1994). It involves ingestion of a labeled test meal with a contrast agent, typically gadolinium tetraazacyclododecane tetra-acetic acid (Gd-DOTA), after a period of fasting. The patient is typically positioned in a right-sided semi-supine position and multiple high resolution images of the entire stomach are recorded (Schwizer et al., 1994, Kim et al., 2000). Computed stomach volumes are reported as proportions of the feed volume.

MRI measures of solid and liquid gastric emptying in adults correlate with that of scintigraphy (Feinle et al., 1999, Schwizer et al., 1994), however this method has not been validated in infants. While MRI can provide direct gastric volume measurements without radiation exposure, it is expensive, is not widely available, and cannot be performed at the bedside (Kim et al., 2000). Further, MRI may contribute to thermal stress as very small infants that are normally housed in incubators, must be removed from the controlled thermal environment for the procedure, and there is evidence that MRI significantly increases infants’ core body temperature (Machata et al., 2009). MRI assessment of brain injury at term corrected age aids the prediction of
neurodevelopmental outcomes in preterm infants (Plaisier et al., 2014) but for gastric emptying studies the frequency and extended duration of data collection increases the likelihood of motion artefact (Edwards and Arthurs, 2011). Sedation is not suitable for infants predisposed to apnoea, and even respiratory movements can cause abdominal scan artefacts (Cahoon, 2011, Kim et al., 2000). MRI may not be feasible for the sick preterm infant, as options for monitoring and ventilation are limited.

While gastric physiology data provided by MRI is detailed and likely to be accurate, several issues including removal of the preterm infant from the neonatal nursery setting for the procedure and its associated impact on infant monitoring and care suggest that MRI is not suitable for monitoring gastric emptying in preterm infants.

1.6.3.1 Scintigraphy

Scintigraphy has been refined to provide a non-invasive, easy to perform and reproducible quantitative measure of anatomical and physiological aspects of gastric emptying in adults and children (Moreira and McQuiggan, 2009, Heyman, 1995). Developed as a non-invasive measure of gastric emptying over 30 years ago, it is widely considered to be the gold standard against which other methods have been validated (Barbosa et al., 2005, Ghoos et al., 1993, Gomes et al., 2003, Maes et al., 1995, Naslund et al., 2000, Sutton et al., 1985). However, standardization of test meals, test procedures and data acquisition methods are lacking for both adult and paediatric populations (Seok, 2011, Urbain and Charkes, 1995, Heyman, 1995).

The method involves ingestion of a test feed that is labeled with a radioactive marker such as technetium-99m and tracked using a gamma camera to image and measure the amount of radioactivity emitted from the stomach. Immediately post feed and serial postprandial measures are used to report proportions of the immediate post-feed value, which correspond with retained feed proportions. Data are also used to construct a gastric emptying curve and to calculate \( T\frac{1}{2} \) (Donohoe et al., 2009).

Scintigraphy has been trialed in preterm infants (n=10) within the neonatal nursery setting using a transportable gamma camera (Bode et al., 2004). A low isotope dose was given to minimize radiation exposure, resulting in long data acquisition times
Consequently the gamma camera was positioned within centimetres of the abdomen almost continuously for the first postprandial hour thus restricting infant movement. Also, researchers reported erroneous measurements resulting from scatter radiation emitted from the labeled meal that had passed into adjacent intestine. The sheer size of preterm infants makes this issue particularly problematic.

Scintigraphic studies of liquid gastric emptying in adults use water as the test meal (Seok, 2011). The preterm infant’s recommended diet of breastmilk is relatively high in fat (Jensen, 1995) and with variable composition (Ballard and Morrow, 2013), emptying may differ between feeds so it does not provide a suitable standardized test meal. Importantly, scintigraphy does not account for the rapid emptying that occurs during delivery of a liquid meal, resulting in overestimation of the rate of gastric emptying and $T_{1/2}$ (Lin et al., 2000). Attempts to minimize exposure to radiation, and scatter radiation are particularly difficult issues for infants, suggesting scintigraphy in its current form is largely unsuitable for use in the preterm population.

**1.6.3.2 Antral cross sectional area (ACSA)**

Measurements of transverse ultrasound images of the gastric antrum are used to calculate the antral cross sectional area (ACSA), providing a proxy measure that correlates with gastric volumes in adults (Bolondi et al., 1985, Duan et al., 1993, Darwiche et al., 1999, Perlas et al., 2009, Perlas et al., 2013) and preterm infants (Ewer et al., 1994). Graphed ACSA measurements are assumed to reflect patterns of gastric emptying. The time taken for the ACSA to reduce to 50% of its maximum area provides an estimation of $T_{1/2}$ that has been used to evaluate emptying in studies of preterm infants (Newell et al., 1993, Riezzo et al., 2000, Riezzo et al., 2001, Riezzo et al., 2009).

This method is well suited to the preterm population as diagnostic ultrasound is a well-accepted methodology that can be performed at the bedside with minimal risk to the infant. However the ACSA measurement provides a proxy rather than a direct measure of gastric volume, and both intragastric gas and postprandial body positioning can influence ACSA measurements in newborn infants (Tomomasa et al., 1996). The ACSA method has not been validated in the preterm population, and anomalous patterns of ACSA change observed in graphed serial ACSA measurements call into question its reliability (McClure and Newell, 1996, Newell et al., 1993).
1.6.3.4 Direct ultrasound method

Direct ultrasound methods of measuring stomach volume have been trialed in adults including the construction of three dimensional (3D) representations of the stomach from a series of parallel cross-sectional image ‘slices’ (Bateman and Whittingham, 1982), and more recently 3D ultrasonography (Gentilcore et al., 2006, Hata et al., 2010). Presently bedside 3D ultrasound is not widely available, and neither of these methods has been validated.

Estimations of stomach volume from gastric ultrasound image measurements has been trialled in young healthy infants (n=6) and those with gastric emptying disorders or requiring specialist infant formula feeds (n=14) (Lambrecht et al., 1988). The volume calculation was based on the assumption that the infant stomach is spheroid in shape, and results used to report retained feed proportions at serial time points. Image acquisition took only a few minutes and the procedure was well tolerated. The direct ultrasound method has been validated against scintigraphy in 10 infants and children (ages 6 months to 3 years) (Gomes et al., 2003). Its use in preterm infants is appealing as bedside ultrasound is an accessible, non-invasive, low risk and physiological method.

Despite the availability of over ten different methods of monitoring gastric emptying, none to date have provided a validated, safe and accessible method for use within the preterm population (Table 1.1).
Table 1.1 Features of gastric emptying monitoring methods for infants

<table>
<thead>
<tr>
<th>Features</th>
<th>Intubation</th>
<th>Gastric aspiration</th>
<th>Dye dilution</th>
<th>Indirect</th>
<th>Paracetamol test</th>
<th>Isotope breath test</th>
<th>Epigastric impedance</th>
<th>Imaging</th>
<th>Scintigraphy</th>
<th>MRI</th>
<th>ACSA</th>
<th>Direct ultrasound</th>
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1.7 Intrinsic influences on gastric emptying

Growth and physiological maturation of the gastrointestinal tract continues throughout gestation and in the months following birth (Lai et al., 2014, Liang et al., 1998, Neu, 2007, Sase et al., 2002, Sase et al., 2005a), with gastric and duodenal motility contributing to the regulation of gastric emptying of liquid meals (Horowitz and Dent, 1991). Functionally immature gastro-duodenal motility has been observed following preterm birth (al Tawil and Berseth, 1996, Ittmann et al., 1992, Riezzo et al., 2000) and is associated with the slower gastric emptying observed in infants born <34/40 (Neu, 2007, Riezzo et al., 2000, Cavell, 1982, Boccia et al., 1999). Gastric emptying is influenced by maturation of gastro-duodenal motor responses that may be related to gestational age as well as postnatal age of the preterm infant.

1.7.1 Birth gestation

While delayed gastric emptying and the associated signs of feeding intolerance are a common problem in preterm infants, it is not known whether infant gestation
influences gastric emptying in feed tolerant preterm infants (Neu, 2007). To date only two studies have specifically compared measures of gastric emptying between preterm infants of differing gestations (Cavell, 1981, Riezzo et al., 2000).

Cavell compared gastric emptying of breastmilk and infant formula in preterm (n=11) and term infants (1–6 months, n=17) (Cavell, 1981, Cavell, 1982). This is the first published comparison of preterm gastric emptying with that of more mature infants. The marker dilution technique was used with PEG to calculate T½ and volumes of milk emptied per 0.1m² body surface area. Preterm infants of 28–34/40 and 1 – 9 weeks old were fed intragastric tube feeds of ~22 mL/kg while the term infants received feeds of ~32 mL/kg. For both groups, a biphasic pattern of emptying was typically observed for breastmilk feeds while a linear pattern was usually observed for formula feeds. The T½ were consistently shorter for preterm infants and is likely due to the much lower feed volumes, with term infants’ feed volumes typically >100 mL higher (based on calculations of reported weights and mL/kg (Cavell, 1981, Cavell, 1982)). In the first postprandial hour, volumes emptied per 0.1 m² body surface area were for preterm vs. term infants: breastmilk; 19.4±4.5 mL and 29.5±4.6 mL respectively, and formula 13.8±2.8 mL vs. 22.7±4.2 mL respectively. These results reflect slower emptying of formula, and more rapid emptying in infants aged 1 – 6 months likely associated with rapid maturation of gastro-duodenal motor activity in the first two postnatal months. Comparison of gastric emptying of preterm infants to term infants in the first postnatal month may be more useful.

Riezzo et al. (Riezzo et al., 2000) conducted simultaneous monitoring of ACSA and EGG, a measure of electrical activity of the gastric smooth muscle but not motor activity (Huerta-Franco et al., 2012), to determine gastric emptying and myoelectrical activity in preterm and term infants. Infants were grouped according to birth gestation and were mean (range) 30.3/40 (28 to <32/40, n=12); 33.3/40 (32 to 36/40, n=11) and 37.8/40 (36 to 40/40, n=10). Infants born 28 – 32/40 had longer T½ and significantly higher proportions of tachygastria than those born >32/40. Further, advancing gestational age was associated with a small but significant increase in the proportion of normal gastric slow waves (p<0.05). Clarification of the association between slower gastric emptying and immature myoelectrical activity observed in very preterm infants is needed.
1.7.2 Postnatal maturation

Several studies have examined the effect of advancing postnatal age on gastric emptying in preterm infants (Boccia et al., 1999, Carlos et al., 1997, Pozler et al., 2003, Riezzo et al., 2009) although few have examined those born very preterm. Gastric myoelectrical activity matures significantly in the first eight postnatal weeks, with the increasing proportions of postprandial (normal) gastric slow waves and exposure to enteral feeds thought to facilitate emptying (Liang et al., 1998).

To determine the effect of increasing postnatal age, gastric emptying curves and T½ were compared between sequential feeds in preterm infants born at 26/40 (23–29/40) (Carlos et al., 1997). ACSA measurements were used to study emptying of ‘early’ partial enteral feeds of ≥20 mL/kg/day (mean age 12 days, n=23), full enteral feeds of 155 mL/kg/day (mean age 23 days, n=23), and full feeds at ≥32/40 weeks corrected gestational age (CGA, n=16). Only 44% (14/32) of infants received the same feed type i.e. breastmilk or preterm formula at each study feed. A curvilinear pattern of emptying was observed, which is comparable to the emptying pattern of liquid meals observed in children and adults (Lin et al., 2000, Siegel et al., 1988, Urbain and Charkes, 1995). A trend to shorter T½ (18±13 min vs. 14±13 min, early and full feeds, respectively) that the authors suggested was an effect of postnatal maturation.

Riezzo et al. studied preterm infants (n=18) at 3, 7, 14 and 30 postnatal days using serial ACSA measurements and EGG to determine whether postnatal age influenced T½ and myoelectrical activity (Riezzo et al., 2009). Infants born at 34/40 (28–36) were fed preterm formula ranging from 30 to 180 ml/kg/day. When compared to day 3 there was a trend towards faster gastric emptying (p=0.08) and increased postprandial gastric electrical activity (p=0.02) on day 7, but subsequent measures were similar across the first postnatal month. Gestational age of study infants was skewed towards the higher end, so the results therefore cannot be applied to very preterm infants, but suggest that for infants born ≥34/40, advancing postnatal age does not impact gastric emptying.

Pozler (2003) evaluated the use of the 13COABT in newborn preterm infants (n=16) by comparing T½ at three time points between 11–227 hours of age. Infant birth gestation was 34/40 (31–37). Test feeds of 13C primed pasteurized breastmilk were studied when the feed volumes were <7 ml/kg, 7–13 ml/kg and 10–19 ml/kg,
with the same body position maintained for each test feed. As calculated T½ were similar between feeds (p=0.68), the authors suggested that increasing age during the first postnatal week does not influence gastric emptying. The study sample included only three infants born ≤32/40, and one of these infants had an extremely long T½ for the first study feed so these results cannot be applied to very preterm infants.

To determine postnatal changes in T½ and gastric myoelectrical activity, weekly simultaneous serial ACSA and EGG measures were studied in infants born 25 – 28/40 (n=12) and compared with that of term infants (n=10) (Boccia et al., 1999). Both T½ and total duration of emptying of the formula test meal reduced between the first study feed at 25–28/40 and 35/40 CGA (T½: 82 min (80-95) vs. 45 min (35–75, p<0.01); total duration of emptying; 120 min (100-175) vs. 110 min (92-120) respectively, p<0.01). While the wide range of durations of emptying suggest that the stomach was often emptied well in advance of the next feed, the correlation between total emptying time determined by ACSA and actual emptying of the stomach has not been established. The T½ was similar between infants at 35/40 CGA and term infants, suggesting that for extremely preterm infants, postnatal development of gastric emptying and electrical activity continues until mature patterns are reached at around 35/40 CGA.

Current evidence indicates that the rate of gastric emptying in infants born >34/40 is similar to that of term infants. The limited data available for very preterm infants suggest that maturation of myoelectrical patterns and gastric emptying occurs with increasing postnatal age.

By necessity, studies of the effects of birth gestation and postnatal age on gastric emptying involve the comparison of feeds of differing volumes as larger feed volumes are prescribed with increasing infant weight and with advancement of enteral feeding. Feeds of larger volume likely have an initial faster emptying rate than those of smaller volume (Kwiatek et al., 2009, Schmitz et al., 2012) that is not reflected in T½, yet this measure is compared in all of the published maturation studies. Further, consistency of feed type, feed composition and infant body position may impact gastric emptying but were potentially not consistent between study feeds (Carlos et al., 1997, Pozler et al., 2003). Use of a more sensitive measure of emptying over time such a retained feed proportions, and statistical analysis methods that account for
potential influences on gastric emptying such as mixed effects modelling will assist in clarifying the effects of maturation on preterm gastric emptying.

1.8 Extrinsic influences on gastric emptying

1.8.1 Feed volume

Gastric emptying of liquids is reported to be mono-exponential in adults, whereby a constant proportion of the remaining stomach residual volume empties over time (Mariani et al., 2004). This is congruent with observations of feeds of higher volume emptying at a faster rate (mL/min) during the early postprandial period than those of a lower volume (Kwiatek et al., 2009, Schmitz et al., 2012). There is limited evidence for the effect of feed volume on gastric emptying in preterm infants.

Ramirez et al. examined intraindividual differences in T½ of intragastric tube feeds of 10 mL/kg and 20 mL/kg of either MOM or preterm formula using 13COABT (Ramirez et al., 2006). There were no significant differences between T½ of the two feed volumes, and the authors concluded that volume alone does not impact gastric emptying in preterm infants. However for feed volumes of 15 mL and 30 mL to attain equivalent T½, the 30 mL feed would have to empty 15 mL in the same timeframe that a 15 mL feed emptied 7.5 mL, indicating a faster initial emptying rate for the larger feed volume. As the T½ provides only a single measure that does not reflect gastric emptying across the postprandial period it is not a suitable measure with which to assess the effects of feed volume on gastric emptying.

While no other published studies have specifically examined the effect of volume on preterm gastric emptying, the results of three studies that have examined feeds of different volumes suggest that feeds of larger volumes empty more rapidly in the early postprandial period. Riezzo et al. (Riezzo et al., 2009) compared intra-individual T½ for formula feeds ranging from 30 to 180 mL/kg/day in preterm infants (n=18) born 34/40 (28–36). Serial ACSA measurements were used to estimate T½ that were similar between feeds, indicating that feed volumes up to 600% higher than the initial test feed emptied at a faster rate to attain T½ similar to that of low volume feeds. The study was conducted throughout the first postnatal month so it is possible that infant maturation may have influenced gastric emptying (Chen et al., 1997),
although current evidence does not support an effect of such large magnitude that would account for the differences observed in this study.

Similarly, Pozler et al. used $^{13}$COABT in infants born at 34/40 (31–37/40; n=16) to determine T½ for three pasteurized donor human milk (PDHM) feeds of different volumes within 9 days of birth (Pozler et al., 2003). Mean T½ were similar between feeds of <7 mL/kg, 7–13 mL/kg and 10–19 mL/kg, indicating that feeds of higher volume had faster early postprandial emptying rates.

Chen et al. examined intra-individual effects of positioning on gastric emptying of MOM feeds of 50 mL/kg/day and 100 mL/kg/day in infants born at 29.8/40 (23.4–35.9/40) (n=35) (Chen et al., 2013). Serial gastric aspiration was performed to determine emptying, with the studies conducted in both prone and supine body positions for each feed volume. As the study focused on the effects of body positioning, statistical analysis did not address the effect of feed volume on emptying. However the published gastric emptying curves clearly demonstrate that feeds of 50 mL/kg/day and 100 mL/kg/day had similar residual feed proportions across the postprandial period reflecting a faster rate of emptying for the larger feed volumes.

![Gastric emptying curves for feeds of differing volumes. Modified from (Chen, Tzeng, Gao, Kua, & Chen, 2013)](image)

**Figure 1.4:** Gastric emptying curves for feeds of differing volumes. Modified from (Chen, Tzeng, Gao, Kua, & Chen, 2013)
The available evidence suggests that early postprandial emptying (mL/min) is faster for feeds of higher volumes although this is yet to be confirmed using methods that accurately measurement gastric emptying over time.

1.8.2 Infant positioning

It is generally considered that the infant’s body position influences the speed of gastric emptying. In the clinical setting, preterm infants with gastro-oesophageal reflux are frequently placed in the prone or right lateral position as a strategy to facilitate gastric emptying and alleviate symptoms of feeding intolerance such as vomiting (Dhillon and Ewer, 2004, van Wijk et al., 2007).

When compared to supine and left lateral positioning of preterm infants, prone positioning is associated with faster gastric emptying in the first 30 minutes after feed delivery (Chen et al., 2013, Hwang et al., 2003, Yu, 1975). However evidence for the effect of positioning beyond the first 30 minutes post feed is conflicting. Chen et al. (Chen et al., 2013) compared the inter-individual effects of prone and supine positioning on postprandial retained feed proportions at 30 min intervals. Prone positioning was associated with feed proportions 15%-25%, lower than that of supine positioning across the postprandial period while Malhotra et al. (Malhotra et al., 1992) found lower final residual volumes and Cohen et al. (Cohen et al., 2004) found no effect of prone positioning. Further when compared to left lateral positioning, right lateral positioning has been associated with lower retained feed proportions at 60 min post feed (Cohen et al., 2004, Omari et al., 2004).
Accuracy of the methods used to investigate the effects of positioning of gastric emptying has not been established, likely contributing to the inconsistent study findings. Differences in gastric emptying observed in different infant positions can be explained by the filling of the stomach during feed delivery. Liquid meals ingested in the prone and right lateral positions fill most of the antrum thus facilitating gastric emptying, whereas in supine and left lateral positions, most of the gastric contents remain in the proximal stomach (Imai et al., 2013). Since body positioning likely impacts gastric emptying it is important that this is accounted for in either study design or statistical analysis when measuring gastric emptying in infants.

1.8.3 Non-nutritive sucking

Non-nutritive sucking (NNS) is facilitated in preterm infants through the use of a “dummy” or pacifier, and is thought to accelerate gastric transit through vagal nerve stimulation (Pinelli and Symington, 2005). Three randomized trials have investigated the effects of NNS on gastric emptying in preterm infants.

Szabo et al. studied paired feeds where preterm infants (n=10) did or did not suck a pacifier for 5 minutes (min) during feed delivery and reported no intra-individual differences in residual gastric volumes aspirated at 10, 20 and 30 min post feed (Szabo et al., 1985). Similarly, Widstrom et al. studied paired intragastric tube feeds where preterm infants (n=8) did or did not have access to a pacifier from 15 min...
prior to 3 hours after feed delivery (Widstrom et al., 1988). For the feeds with pacifier use, the pacifier was offered prior to and during feeding, and thereafter whenever the infant signaled interest in sucking. There were no intra-individual differences in the single measure of gastric residual volume at 3 hours post feed, while the duration of feed delivery was on average 3 min shorter (p<0.02) for feeds with pacifier use. The shorter feed duration may contribute to the perception that NNS aids gastric transit during feed delivery.

Zhao et al. randomized preterm infants to one of two groups; no pacifier use (n=20), or pacifier use (n=18) immediately prior, during, and for 5 min after intragastric tube feed delivery (Zhao et al., 2004). Researchers provided oral stimulation to encourage sucking by moving the pacifier throughout the period of pacifier use. T½ estimated from serial ACSA measurements were shorter for the pacifier group when compared to those with no pacifier (58.3±22.9 min vs. 73.8±17.8 min, p<0.05).

Evidence for the effect of NNS on gastric emptying in preterm infants is unclear, with no effect found in two of three studies. The study conditions and measurements varied between studies with the reported duration of pacifier access ranging from 5 to 195 min, and the actual duration of NNS not reported. Analyses of gastric emptying included comparisons between aspirated residuals at 30 min post feed, between T½ and between final gastric residual volumes. Clarification of the effect of NNS may be achieved through serial postprandial measures of gastric emptying using a validated method, with analysis accounting for the duration of NNS sucking.

Further the vagus nerve contributes to moderation of gastric motility (Deloose et al., 2012) and stimulation of the vagus nerve through oral stimulation is thought to facilitate gastric emptying (Widstrom et al., 1988). However, incomplete myelination of the vagus nerve in infancy (Porges and Furman, 2011), and vagal innervation of pharyngeal and laryngeal muscles but not the buccal and glossal muscles involved in NNS (Matsuo and Palmer, 2008) suggest that facilitation of gastric emptying through NNS is unlikely.

1.8.4 Infant acuity

Acute respiratory distress and intracranial haemorrhage in preterm infants <72 hours of age are associated with delayed gastric emptying with aspirated gastric
residual volumes significantly larger than that of ‘healthy’ preterm infants at 30 min post feed (Chen et al., 2013, dos Santos Mezzacappa and Collares, 2005, Yu, 1975). As both studies assessed only a single time point, postprandial effects of newborn disease on emptying subsequent to 30 min post feed are not known. Treatment of respiratory distress with nasal continuous positive airway pressure (CPAP) increases superior mesenteric artery blood flow at 30 min post feed (Havranek et al., 2007) with gastric emptying reported to be faster compared to infants not requiring respiratory support (Gounaris et al., 2004). Knowledge of the effects of infant acuity and medical management on preterm gastric emptying is still evolving and should be considered in gastric emptying studies.

1.8.5 Medications

Screening for retinopathy of prematurity requires the administration of mydriatic eye drops to facilitate ophthalmic examinations through pupil dilatation. While systemic absorption is minimal, side effects and complications such as transient paralytic ileus have been reported (Lim et al., 2003). An increased incidence of feeding intolerance in the 24 hours following eye examinations has been attributed to the use of mydriatics and/or the stress of the examination itself (Hermansen and Sullivan, 1985). There is conflicting evidence as to the effect of mydriatics on delayed gastric emptying. Bonthala et al. compared gastric residual volumes at 20 min and 2 hours post feed and antro-duodenal activity in preterm infants (n=11) for paired feeds given prior to and following mydriatics and eye examination (Bonthala et al., 2000). The average retained feed proportions at 20 min and residual aspirates at 120 min were significantly higher (57%±5 vs. 43%±5, p<0.05, 1.2 mL±1.5 vs. 2.1 mL±0.5, respectively), and both fasting duodenal activity and the incidence of expected motor responses to feeding significantly reduced (both p<0.05) after mydriatics and eye examination. In contrast, Bózsa et al. compared gastric emptying before and after mydriatics and eye examination using sonographic measures of gastric volume at 30 and 60 min post feed in 20 preterm infants, and found that paired stomach volume measurements were similar. The conflicting findings may be explained by differences in methods used to evaluate gastric emptying, and in the type and dose of mydriatic drops used.

Attempts to facilitate gastric emptying in feeding intolerant preterm infants
through the use of gastrointestinal prokinetic agents have had mixed results, with unconvincing evidence of shorter gastric emptying time. Further, most of the medications are associated with risks or side effects that limit their use in the neonatal population (Young, 2010).

Evidence for the effects of cisapride on gastric emptying in preterm infants is conflicting with treatment reported to result in $T_{1/2}$ significantly shorter (n=15) (Costalos et al., 2000), similar to (n=16) (Barnett et al., 2001), and significantly longer than placebo (n=10) (McClure et al., 1999). Importantly, cisapride has been associated with cardiac repolarization abnormalities such as prolonged QTc interval, with very preterm infants at particularly high risk (Dubin et al., 2001). Consequently access to cisapride has been restricted in the paediatric setting since 2000 (Maclennan et al., 2010).

Erythromycin is an antibiotic with a chemical structure similar to motilin that effects gastric motor activity whereby doses of 3mg/kg stimulate migrating motor complexes and doses of 10–12.5mg/kg stimulate antral contractions (Young, 2010). Evidence for the effects of erythromycin on gastric emptying is lacking. A systematic review on the use of erythromycin for feeding intolerance concluded there is insufficient evidence to support the use of low dose erythromycin for prophylaxis against feeding intolerance, but use at higher doses as a rescue strategy facilitates a faster transition to full enteral feeds (Ng, 2009). Erythromycin is associated with an increased risk of hypertrophic pyloric stenosis in young infants and may contribute to bacterial resistance, so short term use is recommended in the absence of other alternatives (Maheshwai, 2007).

Metoclopramide increases peristalsis and facilitates coordination of gastroduodenal motor activity (Young, 2010). While used to treat feeding intolerance, there is limited evidence regarding its effect on gastric emptying in preterm infants. Hyman et al. found the rate of gastric emptying more than doubled to that prior to treatment in infants following abdominal surgery (n=6), but no effect on infants with gastroparesis related to prematurity (n=7) (Hyman et al., 1988). Another study compared retained feed proportions at 30 min post feed for feed pairs studied before and after metoclopramide dosing in preterm infants (n=15) (Blumenthal and Costalos, 1977) and found no effect on gastric emptying. The effectiveness of metoclopramide in
facilitating gastric emptying in preterm infants is not clear. Further adverse effects of metoclopramide include extrapyramidal effects, irritability, drowsiness, methaemoglobinaemia and galactorrhoea (Young, 2010), with a case report of dystonia in a preterm infant recently published (Eras et al., 2013). Metoclopramide is therefore used with caution as toxicity of this drug in infants is yet to be established (Hibbs and Lorch, 2006).

Domperidone is a prokinetic drug that increases the rate of gastric emptying by enhancing antro-duodenal motor activity and peristalsis. One published study of feed pairs before and after domperidone treatment was carried out in feeding intolerant preterm infants (n=22) (Gounaris et al., 2010). The mean T½ calculated from serial ACSA was significantly shorter with domperidone treatment (48±24 min vs. 68±26 min, p<0.008). Unfortunately a potential adverse effect of domperidone is delayed cardiac ventricular repolarization with lengthening of the QTc interval. Use of the drug is currently prohibited in the United States and restricted in other countries due to safety concerns (Young, 2010).

Gut microbiota play a role in the maturation and modulation of gastrointestinal sensorimotor functions. It is thought that probiotics may assist in normalizing gut colonization and function in the preterm infant (Di Mauro et al., 2013), although there is currently insufficient evidence to support the use of probiotics to promote feeding tolerance (Mihatsch et al., 2012). Indrio et al. have examined the effect of probiotics and prebiotics on preterm gastric emptying and myoelectrical activity in two separate studies. One study found that in three groups of ten preterm infants receiving breastmilk feeds, formula plus placebo, and formula plus the probiotic \textit{L. reuterii} post-test gastric myoelectrical activity was similar between groups, but the probiotic group had significant reductions in fasting antral area and ACSA from immediate post feed to 2 hours post feed. In fact, the probiotic group’s post-test measures were similar to that of the breastmilk group. The other study found that infants fed with formula supplemented with oligosaccharide prebiotics had a higher proportion of efficient electrical activity that supports peristalsis and a shorter T½ than those fed formula only. While the results of Indrio’s research is encouraging, larger studies are needed to evaluate the effectiveness of the doses, timing and safety of various prebiotics and probiotics strains in facilitating gastric emptying.
1.9 Nutrient and biochemical influences on gastric emptying

Gastric emptying is delayed with triggering of the ileal brake whereby detection of specific end products of fat, carbohydrate and protein digestion results in inhibitory feedback. This occurs through the activation of neural pathways and secretion of gastroenterone such as CCK, GIP, GLP-1 and PYY. Gastroenterone secretion varies according to the physicochemical structure and load of the end products of nutrient digestion, with fatty acids considered to be the most potent triggers of the ileal brake in adults (Shirra, 2004; Shin, 2013).

1.9.1 Energy Density

Evidence from adult studies indicate that liquid meals of higher energy density empty more slowly than those of lower density irrespective of the biochemical composition of the meal, with effects observed across a wide range of energy densities (3–60 kcal/30mL) (Kwiatek et al., 2009, Calbet and MacLean, 1997). However, the effect of energy density on gastric emptying in infants is unclear due to limited and inconclusive evidence.

Ramirez et al. compared gastric emptying of formula or breastmilk with energy densities of 5, 10 and 20 kcal/30mL and volume 10ml/kg in 10 preterm infants (Ramirez et al., 2006). The T½ and gastric emptying coefficients, estimated using the $^{13}$COABT, were similar between feeds. Dilution of test feeds was based on the assumed energy density of 20kcal/30mL for undiluted breastmilk (Ramirez et al., 2006), yet a range of energy densities is observed in term breastmilk (21 kcal/30mL; range 19-27.6) with higher densities seen at earlier gestations (25.7 kcal/30mL; range 21.3-29.7) (Ballard and Morrow, 2013). Therefore the actual energy density of breastmilk can vary from 21% lower to 49% higher than the assumed value, likely confounding the results of studies based on assumed values.

Siegel et al. compared gastric emptying of 22mL/kg feeds of distilled water and preterm formula of 6.5, 13, 20 and 24 kcal/30mL in 10 preterm infants (Siegel et al., 1984). Serial postprandial aspirates were recorded every 20 min using the double dilution technique. Intra-individual retained feed proportions were higher with each incremental increase in energy density, with the exception of 20 kcal/30mL and 24
kcal/30mL feeds whereby retained feed proportions were similar except at 80 min post feed.

Several factors may explain the differing results of these two studies. Ramirez studied infants born at 25–30/40 that had achieved only 50% enteral feeds. While postnatal age was not reported in this study, it is likely that the infants were <2 weeks of age. In contrast, Siegel studied preterm infants born 25–35/40 with postnatal ages ranging 1–12 weeks. It is therefore possible that postnatal maturation and exposure to enteral feeding influenced the emptying of feeds of differing energy densities.

Comparison of feeds of differing compositions using unvalidated measures of gastric emptying, and assumptions about the energy density of breastmilk are likely to have contributed to conflicting results. Feeding of diluted breastmilk or formula feeds is not a commonly sustained practice, and so current evidence regarding emptying of feeds of expected and lower energy densities does not inform clinical practice. To date there are no published studies that have examined the effects of differing energy densities (based on the biochemical composition of test feeds) on preterm gastric emptying.

1.9.2 Carbohydrates

There is limited evidence that carbohydrates influence gastric emptying in both adults and infants with the effect varying between carbohydrates of different chemical structures. Products of carbohydrate digestion trigger GIP and GLP-1 release, with a more rapid release of GLP-1 compared to that of fatty acids (Mansour et al., 2013) potentially resulting in faster inhibition of gastric emptying.

Gastric emptying of glucose polymer and glucose solutions of similar volume and osmotic concentration was studied in adults (n=6) using a modified double sampling aspiration technique (Vist and Maughan, 1995). Following nasogastric tube delivery of test meals, gastric aspirates were sampled every 10 min over the first postprandial hour. Retained feed proportions were lower for glucose polymer solutions than for glucose solutions at 10 min post feed (58% vs. 66%, p<0.05) but not at subsequent time points, suggesting a small but clinically irrelevant effect of glucose polymer on emptying.
Gastric emptying of formula feeds with differing carbohydrate and fat sources were studied in preterm infants (n=11) using the dilution marker technique to measure postprandial aspirates every 20 min for 3 hours (Siegel et al., 1985). Feeds with glucose, glucose polymer or lactose as carbohydrate source were tested with medium chain triglyceride (MCT) and long chain triglyceride (LCT) as the fat sources. Retained feed proportions of glucose polymer solutions with MCT and LCT were 6.8% and 12.8% lower than for glucose solutions respectively at 40 min post feed. Similarly, retained feed proportions of glucose polymer solutions with MCT and LCT were 7.1–9.6% lower than for lactose solutions at 60 and 80 min post feed. Although statistically significant, the magnitude of the effects was small. While feeds of differing carbohydrate source were matched for fat source, other nutrients differed between feed types. The range of osmolar concentration for test feeds was 140–459mOsm/l/kg water and as gastric emptying is influenced by feed osmolality (Pearson et al., 2013), it is possible this confounded the results.

Limited evidence from both adult and neonatal studies suggests that glucose polymer solutions empty slightly faster than glucose and lactose solutions, although the effects are sporadic. As fortified breastmilk rather than formula is the recommended source of nutrition for very preterm infants (American Academy of Pediatrics, 2012, Eidelman, 2012), use of a validated measure in the examination of the effect of carbohydrate on emptying of breastmilk feeds will be valuable to clinical practice.

Lactose is the predominant carbohydrate source in breastmilk, with mean concentrations 67–78 g/L for mature breastmilk and 57–76 g/L in the first 8 weeks after preterm birth (Ballard and Morrow, 2013, Narang et al., 2006). Khan studied the effects of breastmilk composition on gastric emptying in breastfed infants (ages 5–20 weeks, n=27) using serial sonographic measures of stomach volumes and found no association between lactose concentration and gastric emptying (Khan, 2012a). Lactose concentrations rapidly increase with the transition from colostrum to breastmilk, and average concentrations are higher in preterm milk than in term milk (75±6 vs. 62±9g/L) during the first eight postnatal weeks (Ballard and Morrow, 2013, Bauer and Gerss, 2011), yet the effect of lactose concentration on preterm gastric emptying has not been explored.
1.9.3 Protein

Proteins influence gastric emptying through stimulation of the ileal brake with subsequent release of GLP-1, GIP and CCK (Calbet and Holst, 2004, Hall et al., 2003). Knowledge of the influences of physicochemical structure and degree of hydrolysis of proteins on gastroenterone response is emerging (Mansour et al., 2013). While GLP-1 response to protein is the same regardless of degree of hydrolysis, protein hydrolysate is associated with higher serum GIP levels in the first 20 min of gastric emptying and 50% greater gastric secretion volume when compared to intact proteins (Calbet and Holst, 2004). An *in vitro* study indicates increased GLP-1 secretion from human intestinal cells in response to increasing concentrations of casein and specific amino acids, but not whey (Chen and Reimer, 2009). In contrast, when compared to casein, adult ingestion of whey is associated with significantly higher plasma concentrations of GLP-1, as well as CCK and GIP. The enterogastrone response is reflected in a steady rate of gastric emptying of the whey solution while the casein solution has a biphasic pattern of emptying (Hall et al., 2003). While differing protein structures result in varied gastroenterone response and subsequent gastric emptying responses in adults, the effect of protein on infant gastric emptying is not clear.

Breastmilk empties from the stomach more rapidly than preterm and term infant formulae (Carlos et al., 1997, Cavell, 1981, Cavell, 1982, Ewer et al., 1994). This may be explained by differences in the physicochemical structures of bovine and human caseins, whereby human casein forms soft flocculent curd that is digested and emptied more quickly than the firm curd associated with bovine casein (Cavell, 1981, Ewer et al., 1994, Van Den Driessche et al., 1999). The protein profiles of preterm and term breastmilk are distinct from that of bovine milk (Armaforte et al., 2010). Up to 976 breastmilk proteins have been identified, with immune function proteins seen in higher proportions than that observed in the human genome (Gao et al., 2012). The average breastmilk protein concentration (19-23g/L) following birth at <34/40 is higher than that of mature term breastmilk (9-12 g/L), and concentrations decrease over the first eight postnatal weeks regardless of birth gestation (Ballard and Morrow, 2013, Bauer and Gerss, 2011). Further the ratio of casein to whey changes during the course of lactation (Lonnerdal, 2003). Human milk proteins differ from bovine milk with regard to biochemical composition and function, and concentrations vary according to
birth gestation and duration of lactation. While gastric emptying of breastmilk is known to be faster than that of bovine based infant formula, it is possible that variable breastmilk protein concentrations may influence emptying.

Khan examined the effect of protein on gastric emptying of breastmilk in term infants (ages 5 to 20 weeks, n=9), and found no association between retained feed proportions and breastmilk concentrations of protein (12±3 g/L), casein (2.7±1.2 g/L) or whey (8±3g/L) (Khan, 2012a). As preterm breastmilk protein concentrations may be 200% higher than that at term (Ballard and Morrow, 2013), and enterogastrone responses differ between term and preterm infants (Kawamata et al., 2014, Siahanidou et al., 2007), Khan's findings cannot be applied to the preterm population.

Aceti et al. examined the effects of breastmilk composition on the incidence of acid gastro-oesophageal reflux episodes in preterm infants (n=17) (Aceti et al., 2009). Breastmilk protein concentrations were 17 g/L (range 12–19.2), with higher concentrations associated with a lower incidence of reflux episodes. The effect of breastmilk protein concentrations on preterm gastric emptying remains to be investigated.

Several studies have investigated the effects of bovine casein and whey protein concentrations and/or hydrolysis states on gastric emptying of infant formulae. The studies are typically confounded by variable osmotic, energy, carbohydrate and fat concentrations and/or sources in test feeds that may also impact on gastric emptying.

Tolia compared gastric emptying of casein-predominant, soy, and whey hydrosylate formulae in term infants (age 1–11 months, n=28) with gastro-oesophageal reflux disease (GORD) (Tolia et al., 1992). Continuous scintigraphy was performed for 1 hour following isocaloric feeds of the same volume on consecutive days. Measures of gastric emptying at 1-hour post feed indicated that whey hydrosylate formula emptied faster than casein predominant formula. Feed compositions of the whey hydrosylate and casein predominant formulae differed with regard to osmolality and fat composition, as well as degree of hydrolysis between protein types, all of which may have impacted gastric emptying.

Billaeau compared gastric emptying of breastmilk (n=23), whey-predominant formula (n=55), casein predominant formula (n=55) and acidified casein predominant
formula in term infants (ages 0–12 months, n=21) whereby approximately half had GORD (Billeaud et al., 1990). Retained feed proportions were estimated at 30 min and 120 min post feed using scintigraphy. No differences were observed between feeds at 30 min post feed, and casein dominant formula had higher feed proportions at 120 min post feed (38%±21) than that of breastmilk (19%±16) and whey dominant formula (25%±18). The authors concluded that higher casein concentrations are associated with slower gastric emptying, however the osmotic concentration of casein predominant formula was 50–280mOsmol/kg higher than the other formulae (data not provided for breastmilk feeds) and the feeds differed with regard to fat and carbohydrate concentrations.

For both studies of the effects of protein composition on gastric emptying in term infants, variations in osmotic concentration and macronutrient composition other than protein may have confounded results. Further, the inclusion of infants with GORD that likely have delayed gastric emptying (Argon et al., 2006) limits the applicability of findings to healthy term infants. With limited and potentially confounded evidence, it is not clear whether differing types and concentrations of bovine protein affect gastric emptying in term infants.

The effect of bovine protein on gastric emptying of infant formula in preterm infants has been addressed in three studies where the impact of casein and whey predominance, and degree of protein hydrolysis were explored.

Gastric emptying of casein-predominant (whey:casein = 18:82) and whey predominant (whey:casein = 60:40) formulae were studied in preterm infants (n=20) in the second postnatal week (Thorkelsson et al., 1994). Biochemical composition and osmotic concentrations of the test feeds were reported to be similar. The T½ and retained feed proportions were estimated at 30, 60, 90 and 120 min post feed using continuous scintigraphy, with T½ also similar between feed types (p=0.75) and insignificant differences of 0-5% calculated between retained feed proportions at each time point. The relative proportions of casein and whey in formula do not therefore appear to influence gastric emptying in preterm infants.

Riezzo et al. investigated the effect of protein hydrolysis on gastric emptying in preterm infants (n=36)(Riezzo et al., 2001) using serial postprandial ACSA
measurements and EGG. Infants were randomized to receive 150 mL/kg feeds of standard formula containing intact whey and casein, or formula containing hydrolysed casein. Mean T½ were similar between hydrolysed and standard formulae (66.4 vs. 74.0) and both serial ACSA measurements and patterns of gastric myoelectrical activity were comparable. The formulae were similar with regard to volume, osmotic concentration and proportions of macronutrients, but differed with regard to concentration and source of protein, carbohydrate and fat, suggesting that differences in the source of macronutrients, and small differences in their concentrations do not significantly impact gastric emptying in preterm infants.

Differences in the gastric emptying of formulae with intact whey and casein proteins as well as partially and extensively hydrolysed whey proteins were also investigated in 17 preterm infants (Staelens et al., 2008). The three formulae were studied in each infant on consecutive days using $^{13}$COABT to estimate T½. The extensively hydrolysed whey protein formula emptied significantly faster than the partially hydrolysed whey protein (46 vs. 55 min, p=0.019) and intact protein formulae (46 vs. 53 min, p=0.008), although the magnitudes of the differences are small. It appears that small differences in carbohydrate and fat sources, and molecular weight distributions of formulae compositions do not impact gastric emptying to an extent that is clinically significant.

Evidence for the impact of protein on gastric emptying in infants is limited, with only three studies investigating the preterm population. The available data is restricted mostly to bovine proteins in infant formulae, which is now almost redundant for very preterm infants in settings where donor human milk is available.

1.9.4 Fat

Fat plays an important role in infant nutrition providing 50% of the exclusively breastfed infant’s total energy intake (Jensen, 1995), which is considerably higher than the 20–35% recommended for adults (Elmadfa and Kornsteiner, 2009). The primary fat source in breastmilk is triacylglycerol, which is hydrolysed by gastric lipase as well as bile salt stimulated lipase (BSSL) and lipoprotein lipase, both contained in breastmilk, to release medium and short chain fatty acids (Fredrikzon et al., 1978, Jensen et al., 1982). Fatty acids in breastmilk and bovine milk are predominantly by mass of carbon
Chapter 1

40

chain lengths 16 and 18, although the fatty acid structures differ between milk types (Innis, 2011). The fat source in bovine-based formulae has been largely replaced with vegetable oil blends to achieve a fatty acid composition similar to that of breastmilk. However, the dietary triacylglycerol structures in infant formulae remain different, with lipolysis of breastmilk triacylglycerol 1.7–2.5-fold higher than that in formula with comparable fatty acid compositions (Armand et al., 1996, Innis, 2011).

Dietary fats are digested by lipase to release fatty acids which in turn slow gastric emptying through the release of GLP-1, GIP, PYY and CCK (Feinle et al., 2003, Marathe et al., 2013, Schwizer et al., 1997). Inhibition of lipase in adults results in faster gastric emptying as fewer fatty acids are released to stimulate receptors of the ileal brake (Feinle et al., 2003), and the degree of inhibition of gastric emptying varies between fatty acids of differing carbon chain lengths and concentrations. Specifically, fatty acids with carbon chains of 12 to 18 are up to four times more effective at slowing gastric emptying in adults than are fatty acids with carbon lengths ≤12 (Hunt and Knox, 1968). A dose response has been noted for oleic acid (carbon chain 18) whereby higher concentrations result in a significantly higher serum PYY levels and subsequent greater inhibition of gastric emptying compared to that associated with lower concentrations of oleic acid (Pironi et al., 1993). There is limited evidence for the effect of fat concentration and fatty acids on gastric emptying in term and preterm infants.

Preterm infants’ gastric emptying of formula feeds with predominantly MCT or LCT fat sources were compared in 11 preterm infants at 33–37/40 CGA (Siegel et al., 1985). The MCT and LCT feeds were isosmotic, and each was examined together with three different carbohydrate sources; lactose, glucose, and glucose polymer. Retained feed proportions were measured every 20 min for 3 hours using the dilution marker technique with polyethylene glycol (PEG). Feeds with MCT emptied faster than feeds with LCT regardless of the type of carbohydrate in the feed, with retained feed proportions 15.3–25.7% lower at every time point (p<0.001). Further evidence however is required using accurate assessment methods to confirm the effect of different fat sources in preterm formulae on gastric emptying.

Significant differences are observed between the degree of lipolysis of fats in breastmilk and formula, and the physicochemical structures of respective amino acids
are dissimilar. It is possible that evidence for the effects of differing fats in infant formula may not apply to breastmilk fed infants. Khan examined the effect of breastmilk fat concentration (3.5±1.1g/L) on gastric emptying in term breastfed infants (ages 5–20 weeks, n=9) using serial sonographic measures of stomach volume to determine postprandial retained feed proportions (Khan, 2012a) and found no relationship between fat concentrations and retained feed proportions (p=0.20).

Breastmilk fat concentrations are significantly higher in preterm breastmilk than in term breastmilk, with concentrations increasing over the first eight weeks after birth at term (2.9±0.3 to 4.9±0.2 g/L) and after preterm birth (2.9±0.1 to 6.8±0.3 g/L, p<0.001) (Bauer and Gerss, 2011). Despite this difference, the effects of breastmilk fat concentration and composition on gastric emptying have not been reported for preterm infants. Digestion and emptying of breastmilk may differ for very preterm infants, and between feeds of MOM and PDHM as a result of potentially impaired lipolysis. The diminished activity of gastric lipase in preterm infants born <34/40 impacts on the release of fatty acids and stimulation of the ileal brake (Jensen et al., 1982). Further, BSSL and lipoprotein lipase are inactivated by pasteurization of human milk (Henderson et al., 1998). It remains uncertain therefore whether these factors influence gastric emptying in preterm infants.

1.9.5 Feed osmolality

The detection of hyperosmolar gastric contents by duodenal osmoreceptors results in delayed gastric emptying and increased gastric secretions, in effect reducing the osmotic load of the gastric contents (Pearson et al., 2013). In adult studies, significant differences in T½ have been demonstrated between isosmotic and hyperosmotic solutions of the same volume: solutions of 1300 mOsmol kg⁻¹ have an average T½ of 130 min compared to 64 min for isosmotic solutions (Vist and Maughan, 1995).

The reported osmotic range for unfortified preterm breastmilk is 263 to 370 mOsmol/L (Thatrimontrichai and Janjindamai, 2009, Yigit et al., 2008). Concern about an association between hyperosmolar feeds and NEC and recommendations that the osmotic concentration of infant feeds should not exceed 400m Osmo/L (Pearson et al.,
2013) have precluded studies of the effects of hyperosmolar feeds on gastric emptying in preterm infants.

Fortification of breastmilk feeds is almost a global standard for very preterm infants. An *in vitro* study indicated that fortification may increase the osmotic concentration of breastmilk above 400 mOsmol/L, exceeding 500 mOsm/L when stored for up to 24 hours in the refrigerator (Kreissl et al., 2013). The increased incidence of feeding intolerance observed in preterm infants fed fortified breastmilk may be explained by delayed gastric emptying secondary to an excessive osmotic concentration of fortified feeds (Ofek Shlomai et al., 2013).

Siegel et al. compared gastric emptying of isoosmolar formula feeds and those with an osmotic concentration of 448 mOsmol/L in preterm infants (*n*=10). Formulae compositions were identical except for the carbohydrate source. A dilution marker technique was used with PEG to measure postprandial aspirates every 20 min for 3 hours. Feeds of higher osmotic feed concentration had higher retained feed proportions throughout the postprandial period and increased gastric secretions 30 min post feed, although these differences were not statistically significant (Siegel et al., 1982). While the authors validated the dilution marker method *in vitro* prior to undertaking the study, in vivo repeatability and reliability have not been established in the infant population. Siegel’s data show twelve occasions where serial retained feed proportions increase rather than decrease over time questioning whether the method was not sensitive enough or whether a physiological process was occurring in response to the presence of PEG or other factors.

Gastric emptying of paired feeds of isoosmolar and low osmotic concentration were compared in preterm infants (*n*=10) using the $^{13}$COABT to estimate T½ (Ramirez et al., 2006). Test feeds included breastmilk and two different formulae, with consistency of feed type maintained between feed pairs. Osmolality was tested and adjusted with sorbitol as necessary to achieve osmotic concentrations of 155 and 310 mOsmol/kg H$_2$O. T½ were similar between paired feeds (89.9±24.1 vs. 93.0±13.3) that were identical except for osmotic concentration. A further comparison between feeds of differing osmotic concentrations and volumes (*n*=7), found shorter T½ of 155 mOsmol/kg and 20 mL/kg feeds compared to 310 mOsmol/kg and 10 mL/kg feeds...
(102.8±15.2 vs. 125.3±35.0, p=0.035), although the estimated total emptying time was not different (Ramirez et al., 2006).

1.9.6 Donor human milk

The recommended source of nutrition for very preterm infants is fortified MOM (American Academy of Pediatrics, 2012, Eidelman, 2012, Quigley and McGuire, 2014). However following very preterm birth up to 72% of mothers are unable to produce the volume of milk required to exclusively meet their infants’ requirements (Carroll and Herrmann, 2013) due to delayed secretory activation and/or subsequent impaired milk production (Cregan et al., 2002, Hill et al., 2005). Pasteurized donor human milk (PDHM) is preferred to infant formula as a substitute where MOM is not available (American Academy of Pediatrics, 2012, World Health Organization and UNICEF, 2003). With increasing global availability of human milk banking, the option of feeding PDHM is becoming more common for many very preterm infants (Landers and Hartmann, 2013).

Many of the health advantages of MOM are conferred by PDHM, including the provision of immune-protective and growth factors to the developing gut mucosa, improved feeding tolerance and protection from NEC (Boyd et al., 2007, Quigley and McGuire, 2014, Simmer and Hartmann, 2009). Short-term weight gain and growth is slower in infants fed fortified PDHM than those fed preterm formula, however infant formula is associated with an increased risk of NEC (Arslanoglu et al., 2013, Colaizy et al., 2012, Corpeleijn et al., 2012). Further, delayed gastric emptying is associated with an increased risk of NEC, and the rate of emptying is significantly slower for formula than for breastmilk. (Ewer et al., 1994, Cobb et al., 2004). The composition of PDHM differs from that of MOM due to variations in birth gestation and duration of lactation as well as the effects of both storage and pasteurization yet no studies have investigated gastric emptying of PDHM (Table 1.2).
Table 1.2 Macronutrient composition and energy densities of preterm, term, and donor breastmilk reported as mean±standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Preterm &lt;33/40 first 8 weeks n=159&lt;sup&gt;a,b,c&lt;/sup&gt;</th>
<th>Term breastmilk &gt;4 weeks n=114&lt;sup&gt;b,c,d&lt;/sup&gt;</th>
<th>Donor breastmilk 3-104 weeks n=3025&lt;sup&gt;e,f,g&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Protein g/L</td>
<td>20.2±4.3</td>
<td>11.6±2.9</td>
<td>11.4±2.7</td>
</tr>
<tr>
<td>Lactose g/L</td>
<td>74.1±5.4</td>
<td>74.8±13.4</td>
<td>75.3±14.9</td>
</tr>
<tr>
<td>Fat g/L</td>
<td>42.3±8.5</td>
<td>33.9±7.6</td>
<td>34.3±10.1</td>
</tr>
<tr>
<td>Energy kcal/30mL</td>
<td>25.5±0.3 n=137&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>22.8±0.2 n=73&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>21.4±0.3 n=523&lt;sup&gt;e,f&lt;/sup&gt;</td>
</tr>
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</table>

Modified from (a: (Bauer and Gerss, 2011), b: (Narang et al., 2006), c: (Hsu et al., 2014), d: (Nommsen et al., 1991), e: (Wojcik et al., 2009), f: (Vieira et al., 2011), g: (Michaelsen et al., 1990).

Breastmilk macronutrient concentrations vary widely between women and with birth gestation (Ballard and Morrow, 2013, Kent et al., 2006, Bauer and Gerss, 2011, Hsu et al., 2014, Narang et al., 2006). Compared to mature milk following birth at term, preterm breastmilk concentrations of fat and protein are higher, with an inverse relationship between birth gestation and protein concentration (Bauer and Gerss, 2011, Stam et al., 2013, Underwood, 2013). Further, average preterm protein concentrations decrease by 1.2 g/L per week, and energy values are on average 4.7 cal/30mL higher than term breastmilk across the first 8 postnatal weeks (Bauer and Gerss, 2011). Typically PDHM is sourced from women in established lactation with oversupply following birth at term, although donors may also be sourced after preterm birth (Hartmann et al., 2007, Wagner et al., 2013, Underwood, 2013). The composition of a PDHM feed is likely to vary from that of the recipient infant’s MOM and so it is possible that there are differences in gastric emptying between the 2 different types of milk (Arslanoglu et al., 2013). While unfortified PDHM is associated with improved feeding tolerance when compared to infant formula (Arslanoglu et al., 2013) there are no published studies that compare feeding tolerance or gastric emptying of PDHM and MOM.
1.10 Effects of treatments on breastmilk feed composition

1.10.1 Human milk fortifier

Preterm MOM initially has higher concentrations of macronutrients than that of term MOM, however the concentrations reduce over time (Bauer and Gerss, 2011, Underwood, 2013) and the protein content is inadequate to achieve adequate postnatal growth in the very preterm infant (Kuschel and Harding, 2004). Slow postnatal growth is associated with a higher incidence of morbidities and suboptimal neurodevelopmental outcomes and so the delivery of optimal nutrition is vital (Arslanoglu et al., 2010, Di Natale et al., 2011, Kuschel and Harding, 2004). The use of a commercially prepared multicomponent human milk fortifier (HMF) is recommended to provide adequate protein and micronutrients for the very preterm infant (American Academy of Pediatrics, 2012, Arslanoglu et al., 2013, Quigley and McGuire, 2014).

Fortification not only adds bovine proteins, but acidifies the milk, resulting in changes to feed composition and products of digestion. Infant digestion of bovine proteins in HMF result in serum amino acid profiles that differ from infants fed an exclusive human milk based diet (Polberger et al., 1999). It is not known how various amino acids impact the ileal brake in preterm infants. A reduction in mean pH of breastmilk from 6.8 to 4.5 significantly reduces total protein and lipase while increasing the fat content of breastmilk (Erickson et al., 2013, Maggio et al., 2009, Quigley and McGuire, 2014). Inhibition of anti-infective properties of breastmilk results from the action of iron in HMF (Chan et al., 2007, Quan et al., 1994). Further, HMF increases the osmolar concentration of feeds with reports of concentrations higher than the recommended maximum 400 mOsmol/L (Kreissl et al., 2013, Ofek Shlomai et al., 2013, Pearson et al., 2013). The presence of bovine proteins and potentially hyperosmolar fortified feeds may trigger the ileal brake and there is conflicting evidence as to whether HMF influences gastric emptying.

An exclusive human milk diet with use of HMF manufactured from human milk is associated with a significantly lower incidence of NEC and surgical NEC than a diet of human milk fortified with a bovine based HMF (Sullivan et al., 2010). However as the manufacture of HMF from human milk is limited by cost and availability of human milk, the use of commercially prepared bovine-based HMF is common. The optimal
composition of HMF for preterm infants is yet to be determined, and there are
differences between commercial HMF products with regard to the concentrations and
sources of fats, proteins, carbohydrates and micronutrients (Maggio et al., 2009).
These differences may explain in part the conflicting evidence regarding the effect of
HMF on gastric emptying.

Four studies have examined intra-individual differences in gastric emptying of
unfortified and fortified breastmilk feeds in preterm infants using different HMFs that
vary by macronutrient composition and concentration (Table 1.3) (Ewer and Yu, 1996,
Gathwala et al., 2008, McClure and Newell, 1996, Yigit et al., 2008). Each study used
serial ACSA measurements to estimate T½.

Table 1.3 Summary of studies examining differences in emptying of unfortified (UF) and fortified (F)
breastmilk in preterm infants.

<table>
<thead>
<tr>
<th></th>
<th>Ewer &amp; Yu(^a), 1996</th>
<th>McClure &amp; Newell(^b), 1996</th>
<th>Gathwala et al.(^c), 2008</th>
<th>Yigit et al.(^d), 2008</th>
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<tr>
<td>Infants n</td>
<td>11</td>
<td>22</td>
<td>25</td>
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<td>20 (6-67)</td>
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<td>HMF Cow &amp; Gate not reported</td>
<td>Lactodex whey:casein 60:40 MCT</td>
<td>Eoprotin intact bovine</td>
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<tr>
<td>fat</td>
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<tr>
<td>carbohydrate</td>
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<tr>
<td>Results</td>
<td>21±4 vs. 48±4 p=0.006</td>
<td>46±6 vs. 47±5 p&gt;0.05</td>
<td>24±5 vs. 24±5 23±5 vs. 24±5 both p&gt;0.05</td>
<td>49±23 vs. 65±36 p&gt;0.05</td>
</tr>
</tbody>
</table>

Modified from (a: (Ewer and Yu, 1996), b: (McClure and Newell, 1996), c: (Hsu et al., 2014), d: (Nommsen et al., 1991).

Ewer & Yu (Ewer and Yu, 1996) examined the effect of FM 85 HMF (Nestlé, Vevey, Switzerland) on emptying in preterm infants (n=11). FM 85 HMF contained whey hydrosylate as the protein source, and maltodextrin as the carbohydrate source.
Feeds fortified with FM 85 emptied more slowly with the mean T½ more than double that of unfortified feeds (mean±SEM: 48±4.0 min vs. 21±3.6 min, p=0.006). A linear pattern of emptying was observed for fortified feeds, compared to a rapid initial emptying phase observed for unfortified feeds. The authors attributed the slower emptying of fortified feeds to higher caloric content with triggering of the ileal brake although feed compositions and energy densities were not measured.

Gathwala et al. (Gathwala et al., 2008) studied the effect of Lactodex (Raptakoss Brett & Co. Ltd., Bombay, India) HMF on gastric emptying in preterm infants (n=25) in the first and third postnatal weeks. The HMF whey:casein ratio was 60:40, maltodextrin provided the carbohydrate source and MCT the fat source. Unfortified and fortified feeds had similar T½ in the first and third postnatal weeks (24.0±5.0 min vs. 24.4±5.1 min; 22.8±4.6 min vs. 23.6±4.9 min) that were similar between time points suggesting no effect of fortification or advancing postnatal age.

Gastric emptying of paired feeds of unfortified and Human Milk Fortifier (Cow & Gate) fortified MOM was compared in 22 preterm infants, using the same batch of MOM to prepare each infant’s study feeds (McClure and Newell, 1996). Estimated T½ were similar for unfortified and fortified feeds (mean±SE; 46±5.5 vs. 47.2±5.4 min) suggesting no effect of HMF on gastric emptying.

Intra-individual differences in emptying of unfortified MOM, half strength fortification and full strength fortification were examined in preterm infants (n=20) using Eoprotin HMF (Milupa, Friedrichsdorf, Germany) (Yigit et al., 2008). Eoprotin contains intact bovine protein with dextrin-maltose as the carbohydrate source. Osmolar concentrations were measured for each feed. T½ were similar for unfortified and half strength fortified feeds (49±23 min; 54±29 min), while there was a non-significant trend towards slower emptying for full strength fortified feeds (65±36 min). Osmotic concentrations increased with fortification and were significantly different between unfortified 319±19 (275–371) and half strength fortification 365±25 (310–411, p<0.001) and full strength fortification 440±44 (344–576, p<0.001). The lack of statistical significance of the longer T½ for full strength fortified MOM was attributed to the wide variation in ACSA measures and small sample size. Despite a wide range of osmotic concentrations with a large proportion of full strength fortified feeds
exceeding the recommended limit of 400 mOsmol/L, associations between osmolarity and gastric emptying were not examined.

The incongruous study results may be due to differences in HMF composition, and in breastmilk composition that was not quantified. Specifically, the degree of hydrolysis of proteins, as well as the source and concentration of macronutrients varies between HMF products with possible varying end products of digestion and osmotic concentrations likely to impact differently on the ileal brake. Therefore direct comparisons of emptying of four different HMFs cannot be made. Similarly, as none of the studies measured breastmilk composition the compositions of unfortified and fortified MOM feeds were not accounted for. With a variety of postnatal ages (4–83 days) and birth gestations (25–37/40) in the four studies it is possible that a wide range of breastmilk biochemical concentrations potentially influenced gastric emptying and confounded the results. Also, the studies do not report whether infant positioning was consistent between paired feeds, and with some evidence of an effect of body positioning on gastric emptying (Chen et al., 2013, Cohen et al., 2004, Hwang et al., 2003, Omari et al., 2004, Yu, 1975), this may also contribute to the inconsistencies in reported results.

Finally, unreliable measures may have impacted results with reports of erratic ACSA fluctuations for fortified feeds (Yigit et al., 2008), and gastric emptying curves indicating increases in ACSA during the first 25 postprandial minutes (McClure and Newell, 1996), which is not consistent with gastric emptying. More extensive examination of the effect of individual HMFs on gastric emptying is clearly required.

1.10.2 Refrigeration and frozen storage

Refrigeration and frozen storage of breastmilk are routine and necessary in the preterm nursery setting. For healthy term infants breastmilk may be refrigerated for 3 to 8 days hours, or frozen at -20°C for 6 to 12 months prior to feeding (Eglash, 2010). However guidelines are more stringent for breastmilk intended for hospitalized preterm infants to ensure safety with regard to infection control (Jones and Tully, 2011, Hartmann et al., 2007). There is evidence that both refrigeration and freezing impact the composition of breastmilk.
Refrigeration of fresh breastmilk at 4°C for 96 hours reduces protein by 5% and increases free fatty acids by 3.5%, but does not alter the free fatty acid concentration in pasteurized milk (Slutzah et al., 2010). While fatty acids are known to slow gastric emptying through stimulation of enterogastrone secretion (Feinle et al., 2003, Marathe et al., 2013, Schwizer et al., 1997), it is not known whether the reduced protein concentration or increased fatty acid concentration associated with refrigeration of fresh breastmilk is sufficient to impact gastric emptying in preterm infants.

Few studies have examined the effect of frozen storage at -20°C on breastmilk composition with evidence of reduced fat and energy density (Chang et al., 2012, Garcia-Lara et al., 2012, Garcia-Lara et al., 2013). García-Lara (Garcia-Lara et al., 2012) examined the effect of frozen storage of both manually and ultrasonically homogenized breastmilk on macronutrient composition and energy density. Fresh breastmilk samples were refrigerated for ≤24hr prior to homogenization, sampled for analysis and immediately frozen at less than -20°C. Macronutrient analysis was performed prior to freezing and at days 7, 15, 30, 60, and 90. Progressive reductions in fat concentration and energy density were observed across the study period, with the largest reduction in fat concentration observed after 7 days of frozen storage (manual homogenization 3.9%, p=0.048; ultrasonic 3.6% p<0.002). After 90 days of frozen storage, reductions in fat (14% and 9%, both p<0.001) and energy density (9.8% and 6.1%, both p<0.001) were observed for manually and ultrasonically homogenized samples respectively.

Chang (2012) studied the effect of frozen storage in differing commercial storage bottles and bags on breastmilk macronutrient composition and energy density (Chang et al., 2012). Fresh samples were refrigerated for up to 3 days prior to ultrasonic homogenization, analysed and then stored at -20°C for 48 hours prior to analysis of thawed samples. Macronutrient changes were similar between storage containers. Frozen storage was associated with a reduction in fat concentration of 8.2% to 9.4% (p=0.02) and there was a trend towards lower energy density (p=0.069). Increases in protein (4.4% to 7.7%, p=0.021) and carbohydrate concentrations (0.8% to 1.4%, p=0.001) were also observed.
The macronutrient composition of frozen ultrasonically homogenized pasteurized milk was examined with significant reductions in fat, lactose and energy density observed over time (p≤0.006). Samples were analysed at 30, 60, 90, 120, 150 and 180 days of frozen storage and final reductions in fat (2.7%), lactose (1.7%) and energy (2.2%) concentrations were reported (Garcia-Lara et al., 2013).

Studies of the effect of frozen storage at -20°C vary with regard to the status and processing of breastmilk prior to freezing. Specifically baseline macronutrient analyses were performed on fresh breastmilk that had been refrigerated for 24–72 hours or thawed breastmilk and on pasteurization milk, while the duration of frozen storage ranged from 2 to 180 days. Despite disparate study conditions, all reported an association between frozen storage and fat loss, with reduced energy density at ≥90 days. The reduced fat concentration is thought to be related to lipolysis, rupture of the fat globule membrane through oxidative degradation, and adherence of fat to the storage container (Chang et al., 2012). A reduction in breastmilk fat concentration is clinically relevant as fat contributes approximately 50% of the infant’s energy intake (Jensen, 1995) and persistent reduced energy intake contributes to postnatal growth restriction in the preterm infant (Embleton et al., 2001).

An understanding of the effects of both refrigeration and frozen storage on the biochemical composition of breastmilk is important for hospitalized preterm infants where expressed breastmilk is the main source of enteral feeds. Current evidence indicates that refrigeration and frozen storage significantly change the macronutrient composition of breastmilk and this may potentially influence gastric emptying.

1.10.3 Holder pasteurization

While pasteurization of donor human milk is required to ensure the safety of breastmilk by the reduction of bacterial content however, holder pasteurization changes both the macronutrient and biochemical composition of breastmilk. Holder pasteurization is associated with a reduction in fat concentration compared with fresh raw breastmilk (5.5%, p<0.001) (Vieira et al., 2011) and thawed homogenized breastmilk (3.5%, p value not reported) (Garcia-Lara et al., 2013). The reduction in fat is likely explained by alteration of the fat globule membrane. Lipolysis may contribute in part, although Holder pasteurization significantly reduces the activity of BSSL and
lipoprotein lipase (Arslanoglu et al., 2013, Henderson et al., 1998). It is uncertain whether reduced lipolysis and lower breastmilk fat concentrations with consequent lower energy density (Jensen, 1995, Garcia-Lara et al., 2013) impact the fatty acid load, stimulation of the ileal brake and subsequent gastric emptying.

Reductions in total protein content are associated with holder pasteurization of preterm and term colostrum (both 13%, p<0.001) (Koenig et al., 2005) and fresh raw breastmilk (3.9%, p<0.001) (Vieira et al., 2011) but not thawed homogenized breastmilk (Garcia-Lara et al., 2013).

The reported analysis methods allowed for the measurement of total protein but not specific protein fractions. While holder pasteurization is known to significantly reduce the concentration of immunoprotective proteins such as lactoferrin, lysozyme and secretory immunoglobulin A (Akinbi et al., 2010, Chang et al., 2013), the effect on casein and whey protein fractions is not known. Heating of bovine milk to 80°C changes the physicochemical structure of whey proteins and subsequent whey-casein interactions (Vasbinder and de Kruif, 2003). It is possible that holder pasteurization affects not only the protein content but also the structure of human whey proteins, thus influencing gastric emptying.

The biochemical composition of PDHM differs from that of MOM due to the compound effects of storage and processing as well as macronutrient differences between donor breastmilk and preterm MOM. The additive effects of frozen storage and pasteurization are reported to result in reductions in fat (6.2% to 8%), protein (6%) and energy (5%) (Vieira et al., 2011, Garcia-Lara et al., 2013). For infants that receive predominantly PDHM feeds the reduced nutrient intake and impaired lipolysis resulting from the effect of pasteurization on BSSL and lipoprotein lipase may contribute to growth restriction.

While fat and higher energy content are considered potent inhibitors of gastric emptying in adults, the effects of reduced PDHM fat, protein and energy content concentrations on gastric emptying in preterm infants are yet to be established. It is possible that the lower macronutrient concentrations and energy density may facilitate gastric transit of PDHM. However, it is also possible that the effects of refrigeration, frozen storage, homogenization and holder pasteurization influence the
physicochemical structures of nutrients resulting in interactions with HMF and altered patterns of gastric emptying and curding.

1.11 Intragastric Curding

Milk feeds separate into portions of liquid (whey) and curd (casein) within the stomach of infants and abomasum of cows. Intragastric digestion of casein proteins at a pH of 4.6 causes casein proteins to precipitates out of solution to bind with calcium and form curd (West, 1986). While the whey portion of milk empties rapidly from the stomach, curd is retained in the stomach and its slow intragastric digestion facilitates a steady release of amino acids to the duodenum over time. Curd formation is associated with significantly higher weight gain in non-ruminant calves, with pharmacological inhibition of gastric acid secretion significantly reducing curd formation and subsequent weight gain (Okada et al., 2010). The association between curding and weight gain has not been explored in infants.

Curding is dependent on the availability of casein in an acidic environment. Compared to adults that maintain a stomach pH<4.0 for 88% of the time, postprandial re-acidification of the stomach in preterm infants takes longer with pH<4.0 maintained 59% of the time (Omari and Davidson, 2003). While maintenance of a gastric pH<4.0 from birth facilitates curd formation from milk proteins (Kelly et al., 1993), ingested caseins differ between human and bovine milk. Partially phosphorylated human caseins form a soft, flocculent and easily digestible curd while intact bovine caseins form a firm curd (Miller et al., 1990, West, 1986). Indeed lactobezoar, a rare condition whereby a dense coagulum of intragastric curd causes feeding intolerance and intestinal obstruction, is associated with prematurity and feeds of high casein and MCT content and energy density (Heinz-Erian et al., 2012). Lactobezoar can be clearly identified with abdominal ultrasound but is more difficult to diagnose using standard radiological methods (Naik et al., 1987). Reports of lactobezoar have reduced since a change in infant formula casein:whey ratio from 80:20 to a ‘humanised’ ratio of 40:60 (Schreiner et al., 1982, Heinz-Erian et al., 2012, Stanger et al., 2014), although seven cases of lactobezoar in extremely preterm infants have been associated with the use of HMF containing both casein and whey (Stanger et al., 2014).
The ratio of casein to whey proteins in breastmilk changes over time ranging from 20:80 in early lactation to 50:50 in late lactation (Lonnerdal, 2003) therefore it is possible that breastmilk in late lactation may curd differently to the whey dominant milk of early lactation. Similarly HMF contributes varying proportions of casein and whey that may be partially or extensively hydrolysed and the influence of HMF proteins on milk curding has not been investigated. It is possible that milk protein composition and subsequent curd formation may explain in part the differences in gastric emptying observed between various types of infant milk feeds (Heyman, 1998).

1.11.1 Ultrasound imaging of stomach contents

Serial ultrasound imaging of intragastric contents following milk ingestion has been reported for both pre-ruminant calves and young infants (Khan, 2012a, Miyazaki et al., 2009, Okada et al., 2010). While Khan’s infant subjects were fed breastmilk, and Miyazaki et al. studied young bovine calves fed fresh and adapted bovine milk, both reported a characteristic ‘snow storm’ appearance of fresh milk within 30 min of feeding whereby many echogenic small flecks appear throughout an anechoic area that is thought to represent curd floating in the whey portion of the milk. A similar echogenic appearance has been reported in the adult antrum immediately following ingestion of clear fluids, however the flecks dissipated within minutes of ingestion. It was concluded that the flecks were bubbles of gas that had moved to the gastric fundus (Cubillos et al., 2012). Miyazaki’s euthanasia and subsequent cross-sectional topography of the abomasum of one animal subject confirmed that the observed small flecks were indeed curds floating in the hypoechoic whey portion of the feed (Miyazaki et al., 2009). As the snow storm appearance persists beyond the immediate post feed period in both bovine and infant subjects, it is likely that curd floating in whey is a reasonable interpretation of the snow storm images observed in infants.

Khan described the echogenic appearance of intragastric contents as snow storm (previously described), higher (or more echogenic than the spleen), lower (or less echogenic than the spleen), or mixed (containing areas of both higher and lower echogenicity) reflecting different stages of curd formation or in the case of lower, the absence of curd. The density of curd was rated against that of the spleen, and curd
volume rated as high or low according to whether curd occupied more or less than 50% of the stomach as assessed by longitudinal images of the stomach.

Khan reported that high curd density is observed when most of the feed has emptied from the infant’s stomach (Khan, 2012a). In both infant and bovine studies, a proportionately large curd volume is associated with low fluid volumes reflecting retention of the slowly digested curd and progressive emptying of the fluid whey portion of the feed (Khan, 2012a, Miyazaki et al., 2009).

1.12 Assessment of gastric emptying and curding in preterm infants

Fortified breastmilk is the recommended source of nutrition for preterm infants, providing health and developmental advantages and lower incidence of feeding intolerance and NEC than formula. However, breastmilk composition varies within and between women, as does the addition of HMF, refrigeration, frozen storage and pasteurization. Feed composition impacts gastric emptying and may potentially influence gastric curding of milk feeds. Current methods of measuring gastric emptying have not been validated, or are not suitable for use in the fragile preterm population. Given the impact of gastrointestinal complications on preterm nutrition and growth, an accurate and clinically acceptable method of monitoring gastric emptying would contribute important information to further current knowledge of preterm gastric emptying. Specifically, determination of the repeatability and usual patterns of emptying, and impact of differing feed compositions on emptying and curding in healthy preterm infants will contribute to the nutritional and feeding management of preterm infants.

1.13 Aims

The aims of this research were to validate an ultrasound method of measuring gastric volume in preterm infants, and using serial gastric ultrasound imaging, to investigate the effects of breastmilk feed composition on gastric emptying and curding. In particular, efforts were made to determine how the fat, carbohydrate and protein compositions and energy density of unfortified and fortified breastmilk, MOM and PDHM feeds influenced gastric volumes and the echogenic characteristics of curd throughout the postprandial period.
The specific aims of this thesis were:

1. To validate four sonographic methods of measuring stomach volume and to describe the echogenic characteristics of milk feeds in stable preterm infants at four time points; pre-feed and after 50%, 75% and 100% feed volume delivery.

2. To investigate the repeatability of sonographic stomach volume measurements and echogenic characteristics of stomach contents for paired feeds of the same volume and composition at multiple matched time points i.e. immediately pre and post feed, and at 30 min intervals until the next feed is due (90 min for 2 hourly feeds and 150 min for 3 hourly feeds).

3. To determine the effect of feed macronutrient composition and energy density on postprandial gastric volumes following feeds of unfortified and fortified MOM at multiple matched time points i.e. immediately pre and post feed, and at 30 min intervals until the next feed is due (90 min for 2 hourly feeds and 150 min for 3 hourly feeds).

4. To compare serial postprandial gastric volumes and curding of MOM and PDHM, both unfortified and fortified, and to determine whether the macronutrient composition and energy density of feeds account for any observed differences.
Chapter 2 Validation of Ultrasound Methods to Monitor Gastric Volume Changes in Preterm Infants

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2.1 Abstract

Objectives: To validate four sonographic methods of estimating stomach volume for the purpose of monitoring changes in gastric volume over time, and to describe the echogenic characteristics of milk feeds.

Methods: Twenty-four infants were monitored during a single intra-gastric tube feed, with two ultrasound images of the entire stomach and an image of the antral cross-sectional area (ACSA) before, during, and after the feed. Raw measurements, three stomach volume calculations and ACSA were tested for intra- and inter-rater agreement. Calculated stomach volumes and ACSA were compared to delivered feed volumes, and characteristics of stomach image echogenicity graded at each time point.

Results: Spheroid calculation of stomach volume was the most reliable and valid measure of stomach volume. Fortified breast milk feeds were more echogenic than unfortified breast milk feeds. Residual stomach volumes (median 2.12mL, range 0.59 to 9.27mL) were identified in 18/24 infants.

Conclusions: Direct ultrasound stomach measurement (spheroid) will provide a useful research tool and a potential clinical tool for assessing gastric emptying and feeding intolerance in preterm infants.

2.2 Introduction

Feeding intolerance, a common problem for preterm infants, is characterised by signs of delayed gastric emptying such as large gastric aspirates, abdominal distension and vomiting. These signs may delay the progression of enteral feeding, and so impact on the provision of adequate nutrition as well as the development of
successful oral feeding. Feeding intolerance is associated with necrotising enterocolitis, a gastrointestinal disease with high morbidity and mortality rates (Lin and Stoll, 2006). Current methods for evaluating gastric emptying in the neonatal setting are limited to the assessment of gastric residual volumes through aspiration of the intra-gastric tube, and sequential girth measurements for the evaluation of abdominal distension. Alternative methods of assessing gastric emptying reported in the literature have not been adopted in the clinical setting for many reasons (Bode et al., 2004, Mezzacappa et al., 2005, Nour et al., 1995, van Wijk et al., 2007).

The use of marker dilution techniques and scintigraphic methods has been researched in preterm infants (Mezzacappa et al., 2005, Bode et al., 2004), and neither is suitable for routine clinical use. Marker dilution provides a gross measure of gastric emptying by adding a known quantity of a dye marker to a ‘standard feed’, then aspirating, measuring and replacing the stomach contents at single or multiple time points. This repeated aspiration and replacement of stomach contents is invasive and impacts on normal physiological processes. (Cavell, 1979) Scintigraphic methods, such as the $^{13}$C-Octanoic acid breath test, require exposure to ionising radiation, and are ideally performed after administering a ‘standard meal’ of a set volume and composition; this is not appropriate for preterm infants who are prescribed specific individual feed volumes of varying compositions (van Wijk et al., 2007). In addition, these techniques require complex analyses to estimate gastric emptying (Pozler et al., 2003, Bode et al., 2004). Alternatively ultrasound offers a more physiological and non-invasive technique and has been used successfully to investigate gastric emptying in preterm infants, with antral cross-sectional area (ACSA) shown to correlate with feed volumes delivered to preterm infants (Ewer et al., 1994, Newell et al., 1993).

Published studies report estimated gastric half-emptying times, using the time taken for the ACSA to reduce to half of its maximum area to determine the rate of emptying (Newell et al., 1993, Riezzo et al., 2001). The ACSA technique is limited in that it provides a proxy measure of gastric volume, allowing only for indirect estimates of gastric emptying patterns over time with no indication of actual gastric volumes. The ability to determine gastric volume would enable more sensitive measures of gastric emptying patterns as well as evaluation of gastric residuals in the preterm infant.
Lambrecht et al. (Lambrecht et al., 1988) demonstrated direct measurement of gastric volume in young infants using ultrasound imaging, but did not validate the technique for the preterm infant population. Lambrecht’s method is based on the assumption that the stomach is spheroid in shape. Observations of the fetal stomach in utero(Nagata et al., 1994) and of the stillborn preterm infant’s stomach by the first author at perinatal autopsies indicate that the stomach shape of a preterm infant may be fusiform or an irregular curved spheroid shape. It may be possible to increase the accuracy of existing methods of calculating stomach volume by using combinations of simple geometric shapes to more closely reflect the observed shape of the preterm infant’s stomach.

Ultrasound examination of the stomach provides the opportunity to observe the characteristics of the ingesta as well as its volume and shape. The bright echogenic matter associated with milk feeds in infants is thought to indicate curd or precipitate, and may differ in volume and density between infants and between feeds of differing compositions although this has not been investigated for breast milk feeds in preterm infants (Khan, 2012b).

As for most biological measurements, gastric emptying has an intrinsic variability that influences the reliability and validity of measures. Previous studies of both the ACSA and direct stomach measurement techniques have typically assessed the reliability of the measures used, but not validated these against known volumes such as the feed volume delivered (Ewer et al., 1994, Lambrecht et al., 1988).

2.2.1 Study Aims

This study aimed to determine whether sonography is a reliable and reproducible method for determining gastric volume in preterm infants. Specifically, we compared four methods of estimating stomach volume using a set of calculations based on measurements of sonographic images of the gastric antrum (ACSA) or of the stomach (stomach as spheroid, hemisphere + cylinder, and hemisphere + cone). Calculations were validated against the actual feed volume delivered to determine which method was most accurate.

A secondary aim was to describe the echogenic characteristics of breast milk in the preterm infant’s stomach over the course of delivery of an intragastric tube feed.
2.3 Methods

2.3.1 Participants

Medically stable preterm infants born 28-34 weeks gestation who were receiving full enteral feeds via intragastric tube were recruited from the Special Care Nurseries of King Edward Memorial Hospital, Western Australia. Infants with congenital abnormalities, gastrointestinal disease, or symptoms of feeding intolerance within the previous 24 hours were excluded from the study.

In order to account for individual variability it was determined that a minimum of 20 infants should be recruited. Recruitment continued until usable ACSA and stomach images were available for 20 infants.

The Ethics Committees of the Women and Newborn Health Service and The University of Western Australia gave ethical approval for the study. Parents were provided with verbal and written information about the study and provided signed informed consent for their infants’ participation in the study.

2.3.2 Study Protocol

Each infant was scanned during one feed (duration 14 to 35 minutes), at a time negotiated with parents and staff so as not to disrupt plans for breastfeeding and parent-infant contact. Scanning was scheduled to coincide with the infant’s usual feed time, and was performed at the bedside in the neonatal nurseries using a portable ultrasound machine. After nursing observations and infant cares were attended, the infant was placed in the supine position and the shirt and bedding were loosened to enable access to the chest and abdomen. Sterile gel was warmed for infants in incubators, and used at room temperature for infants in open cots. The intragastric tube was aspirated and tested for acidity as per standard practice to confirm correct tube placement. Feeds were commenced immediately following the pre-feed scan, with infants remaining in the supine position for the duration of the feed. All infants maintained their temperature within normal limits and tolerated the procedure well.
2.3.3 Ultrasound Examination

Serial images of the antrum and stomach were recorded immediately prior to commencement of the feed (0%), and during interruptions to feed delivery when 50%, 75% and 100% of the total volume of the feed had been delivered. Feeds were delivered by gravity, and scanning duration was less than 3 minutes at each time point.

All studies were performed using real time two-dimensional ultrasound using portable ultrasound (Titan hand-carried Sonosite, Australia) with the C11 pediatric transducer. To image the ACSA, the transducer was positioned perpendicular to the abdominal wall, inferior to the xiphi-sternum parallel to the abdominal aorta at the level of the superior mesenteric artery providing a transverse view of the antrum. (Newell et al., 1993) To acquire images for calculation of stomach volume, the transducer was positioned perpendicular to the left lateral chest wall and rotated so that the stomach was both at its maximum diameter and length (longitudinal plane), and a clear view of the spleen was achieved. A second image was acquired with the transducer perpendicular to the longitudinal plane providing a transverse view (Figure 2.1). The intercostal approach was most commonly employed when scanning the stomach of the preterm infants due to the high position of the stomach. The anterior approach was only successful when there was a large volume of milk in the stomach. The position of the transducer was altered as necessary to compensate for infant movement. Only light pressure was exerted to avoid disturbing the infant. Images were recorded on the ultrasound machine and transferred to a lap-top computer for measurement.
2.3.4 Ultrasound Image Analysis

One expert rater and one novice rater conducted image analyses. Prior to analysis, the raters selected the images to be measured; one each of the antrum and the transverse and longitudinal planes of the stomach for each time point (12 images per infant).

Maximum length of the longitudinal stomach image was measured and transverse and anterior-posterior measurements were made of the transverse stomach image (Figure 2.1). Transverse and anterior-posterior measurements were made of the antral image. Each rater made duplicate measurements without access to their previous measurements, or those of the other rater. The images were not measured in sequential order. For each infant, all measurements were made in a single session by each rater.

Measurements were made using ‘Screen Calipers’, V. 4.0 (Iconico Inc. New York, USA) on the raters’ individual lap-top computers. These provide several advantages over traditional on-screen callipers including the ability to use several

Figure 2.1 Ultrasound technique for scanning of the preterm infant’s stomach. The longitudinal and transverse planes for measurement of stomach volume are indicated.
callipers simultaneously, float callipers on the screen, lock rotational angles and stay visible when changing images. This method has previously been used to reliably measure ultrasound images of nipple diameter during breastfeeding. (McClellan et al., 2010)

2.4 Echogenicity of Stomach Contents

Echogenicity of the stomach was assessed by rating the brightness of the longitudinal view of the stomach relative to that of the spleen. Images were classified as having ‘higher’ or ‘lower’ echogenicity if this was consistent across the stomach. Two classifications of variable echogenicity were used. ‘Snow storm’ referred to images with a predominantly fluid filled stomach (anechoic) with many small bright specks interspersed in the fluid. ‘Mixed’ referred to stomach contents appearing as a combination of lower and higher echogenic areas, indicating a mixture of fluid and semi-solid contents (Figure 2.2). Air was not imaged within the infants’ stomachs.

Areas of brightness within the stomach associated with milk feeds were assumed to indicate curd or precipitate. These more echogenic areas were further classified with respect to both density and volume. Density was classified as ‘high’ where the bright echogenic areas appeared bright white (greater than the echogenicity of the spleen), and ‘low’ where they were uneven or grey (equal or less echogenic compared to the spleen). Volume of the bright echogenic area was classified as ‘high’ where it occupied more than half of the stomach area, and low otherwise. Ratings
were made independently by each rater, once only for each longitudinal image, when the first set of measurements were taken.

2.5 **Gastric Measurements**

Measures of gastric volume, including the (proxy) measurement of ACSA, and three different direct stomach volume calculations were tested using the following calculations:

\[ \text{ACSA: } A = \pi \times \frac{\text{antero-posterior diameter}}{2} \times \frac{\text{transverse diameter}}{2} \]

**Stomach Volume Measurements**

1. **Spheroid** (Lambrecht et al., 1988): longitudinal \( \times \) antero-posterior \( \times \) transverse \( \times 0.52 \)

2. **Hemisphere + cylinder**: \( \frac{4}{3}\pi \text{radius}^3/2 + (\pi \text{radius}^2 \text{hemisphere}) \)

3. **Hemisphere + cone**: \( \pi/12 \times \text{transverse}^2 (\text{longitudinal} + \frac{1}{2}\text{transverse}) \)

The spheroid equation is a variation of the standard equation for volume of a spheroid, using diameters rather than radii. The other two equations are approximations to the observed shape of the preterm infant stomach, using a combination of regular solid shape volumes. The equations have been simplified to use diameter measurements rather than radii.

2.5.1 **Statistical Analysis**

All analyses were performed using R 2.9.0 for Mac OSX (R Development Core Team, 2009) (The R Core Team, 2009). Additional packages nlme (Pinheiro et al., 2008b), irr (Gamer et al., 2007b), and lattice (Sarkar, 2008a) were used for linear mixed modeling, intra-class correlations, and lattice plots respectively.

2.5.2 **Intra and Inter-rater reliability**

Reliability of the continuous stomach measurement data were assessed. Comparison of rater responses was made graphically using the method described by Bland and Altman (Bland and Altman, 2002) and analytically using the intra-class correlation (ICC) approach of McGraw and Wong (McGraw and Wong, 2006). The Bland-Altman plots were examined for patterns of disagreement between measurements that related to the magnitude of the measurement.
Intra-rater reliability was assessed using the duplicate measurements, and was calculated separately for each rater. Inter-rater reliability was calculated using the means of the duplicates for each rater. For stomach volumes, the mean of the previously calculated volumes was used. For each measure, measurement bias, ICC for agreement (two-way variance), and 95% confidence intervals (CI) were calculated. Reliability of the two raters was considered to differ if the 95% CIs did not overlap. An ICC >0.8 is considered acceptable for a research setting, and >0.95 acceptable for use in the clinical setting. (Landis and Koch, 1977)

To avoid biasing the assessment of the reliability, images where the stomach was completely contracted (stomach volume = 0 mL), were omitted from the validation of image measurements.

Reliability of the categorical echogenicity and stomach contents measurements were assessed using the Kappa statistic to determine the level of non-chance agreement. For the echogenicity rating, which was the only variable with >2 classification levels, the level of exact agreement was assessed.

Patterns across the increments of the feed were assessed qualitatively for each of the four measures. Relationships between the echogenicity of stomach contents and feed type were assessed for images taken immediately after delivery of the milk feed using Pearson’s Chi-Squared proportion test.

### 2.5.3 Validation of Stomach Volume Techniques

Calculated direct and proxy measures of stomach volume were validated against delivered feed volumes at 50, 75 and 100% of feed by determining whether the discrepancies were significantly different from zero, and if the feed proportion delivered influenced the error. Linear regression and linear mixed effects (with feed as the group and individual baseline errors) were considered as possible models. The linear mixed effect model was only significant for the spheroid calculation, indicating discrepancies were not random.

The first measurements of the novice rater were used for the stomach volume calculations, allowing testing of worst case scenario (likely least accurate) measurements, and reflecting clinical practice where single measurements are most likely to be used.
To determine whether measured pre-feed residuals influenced the measured feed volumes at subsequent time points, linear mixed effects model analysis was employed.

### 2.6 Results

Of the 25 infants recruited, there were 19 complete ACSA and 17 complete stomach data sets. Missing data consisted of: one complete data set lost due to computer failure; five antral images discarded as they were not taken in the correct plane; final ACSA image unusable for one infant; stomach images at 75% and 100% of feed delivery discarded due to poor image quality for one infant. For six infants, the stomach was fully contracted ie. no measurable volume immediately prior to feeding therefore the pre-feed images were not included in calculation of pre-feed residuals.

Characteristics of the infants (13 girls, 11 boys) included in the analysis were mean (range): birth gestation 31 weeks (28-34); birth weight 1620 g (1040-2340) and postnatal age 23 days (2-64). Eight (30%) were small for gestational age (birth weight <10th centile) with the remaining infants appropriately grown at birth.

All infants received breast milk feeds; 16 received their mother’s own expressed breast milk (MOM) and 8 received pasteurised donor human milk (PDHM). S-26 SMA human milk fortifier (Wyeth Nutrition, Baulkham Hills, NSW, Australia) was added to the feeds of 13 infants (7 MOM, 6 PDHM) at the recommended dilution of 1 sachet to 50 mL breast milk. The mean (range) study feed volume was 36 mL (12–55), and the mean prescribed daily feed volume was 157 mL/kg/day (96-184).
2.6.1 Intra-rater reliability of stomach volume measurements

There was a high level of agreement between duplicate measurements performed by the two raters for both antero-posterior and transverse stomach measurements. Other than the antral transverse measurement with ICC >0.95, all measurements had intra-class correlations >0.99 (CI 95%) (Table 2.1). Means and standard deviations of the differences between the duplicates performed by each rater are presented in Table 2.2. The average mean differences were very small – the most extreme was -0.18 mm for the transverse stomach measurement and -0.22 mm$^3$ for one of the calculated volumes, with standard deviations higher for the expert rater. All intra-rater ICC agreement values were within the recommended clinically acceptable threshold of >0.95. (Nunnally and Bernstein, 1994b)

Table 2.1 Intra-rater intra-class correlation coefficients (ICC) for the agreement for repeated image measurements for antral cross sectional area (ACSA) and direct stomach raw measurements and calculations presented as intraclass correlation (95% confidence interval)

<table>
<thead>
<tr>
<th>Raw measurements</th>
<th>Overall</th>
<th>Experienced Rater</th>
<th>Novice rater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antrum.antero-posterior</td>
<td>0.993 (0.991, 0.995)</td>
<td>0.99 (0.984, 0.993)</td>
<td>0.997 (0.995, 0.998)</td>
</tr>
<tr>
<td>antrum.transverse</td>
<td>0.985 (0.979, 0.989)</td>
<td>0.976 (0.963, 0.985)</td>
<td>0.995 (0.992, 0.997)</td>
</tr>
<tr>
<td>stomach.antero-posterior</td>
<td>0.995 (0.994, 0.999)</td>
<td>0.995 (0.992, 0.997)</td>
<td>0.996 (0.994, 0.997)</td>
</tr>
<tr>
<td>stomach.transverse</td>
<td>0.997 (0.995, 0.998)</td>
<td>0.995 (0.992, 0.997)</td>
<td>0.998 (0.997, 0.999)</td>
</tr>
<tr>
<td>stomach.longitudinal</td>
<td>0.997 (0.996, 0.998)</td>
<td>0.996 (0.994, 0.998)</td>
<td>0.998 (0.997, 0.999)</td>
</tr>
<tr>
<td>Calculations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACSA</td>
<td>0.991 (0.988, 0.994)</td>
<td>0.986 (0.977, 0.991)</td>
<td>0.998 (0.996, 0.998)</td>
</tr>
<tr>
<td>spheroid</td>
<td>0.997 (0.996, 0.998)</td>
<td>0.996 (0.994, 0.998)</td>
<td>0.998 (0.997, 0.999)</td>
</tr>
<tr>
<td>cylinder + hemisphere</td>
<td>0.996 (0.995, 0.997)</td>
<td>0.995 (0.992, 0.997)</td>
<td>0.998 (0.997, 0.999)</td>
</tr>
<tr>
<td>cone + hemisphere</td>
<td>0.996 (0.994, 0.997)</td>
<td>0.993 (0.99, 0.996)</td>
<td>0.998 (0.996, 0.998)</td>
</tr>
</tbody>
</table>
Table 2.2 Intra-rater mean differences, standard deviations of the differences and limits of agreement for antral cross sectional area (ACSA) and direct stomach raw measurements and calculations of area and volume.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Expert rater</th>
<th>Novice rater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (SD)</td>
<td>limits of agreement</td>
<td>mean (SD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lower-upper</td>
<td></td>
</tr>
<tr>
<td><strong>Raw measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antrum.antero-posterior</td>
<td>0.05 (0.42)</td>
<td>-0.77-0.87</td>
<td>0.06 (0.52)</td>
</tr>
<tr>
<td>antrum.transverse</td>
<td>0.05 (0.41)</td>
<td>-0.75-0.85</td>
<td>0.07 (0.53)</td>
</tr>
<tr>
<td>stomach.antero-posterior</td>
<td>-0.09 (0.86)</td>
<td>-1.77-1.59</td>
<td>-0.04 (0.95)</td>
</tr>
<tr>
<td>stomach.transverse</td>
<td>-0.10 (0.75)</td>
<td>-1.58-1.37</td>
<td>-0.18 (0.90)</td>
</tr>
<tr>
<td>stomach.longitudinal</td>
<td>0.10 (0.98)</td>
<td>-1.81-2.02</td>
<td>0.15 (1.13)</td>
</tr>
<tr>
<td><strong>Calculations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACSA</td>
<td>0.73 (4.90)</td>
<td>-8.88-10.33</td>
<td>1.10 (6.42)</td>
</tr>
<tr>
<td>Spheroid</td>
<td>-0.11 (1.02)</td>
<td>-2.11-1.90</td>
<td>-0.13 (1.19)</td>
</tr>
<tr>
<td>Cylinder + hemisphere</td>
<td>-0.11 (1.59)</td>
<td>-3.23*-3.00*</td>
<td>-0.22 (1.88)</td>
</tr>
<tr>
<td>Cone + hemisphere</td>
<td>-0.12 (0.95)</td>
<td>-1.97-1.74</td>
<td>-0.19 (1.13)</td>
</tr>
</tbody>
</table>
2.6.2 Inter-rater reliability of stomach volume measurements

Means and standard deviations of the differences between the measurements performed by each rater are presented in Table 2.3. Despite wide limits of agreement between raters’ measurements, there was no evidence of bias. The most extreme mean difference between final measurements was 0.89 mm (antrum.antero-posterior, SD 2.49 mm).

Table 2.3 Inter-rater mean differences, standard deviations of the differences and limits of agreement for antral cross sectional area (ACSA) and direct stomach measurements. Limits of agreement are mean±(1.96* sd) for 95% certainty.

<table>
<thead>
<tr>
<th></th>
<th>mean (sd)</th>
<th>limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>lower-upper</td>
</tr>
<tr>
<td><strong>Raw measurements (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antrum.antero-posterior</td>
<td>0.89 (2.49)</td>
<td>-3.99-5.77</td>
</tr>
<tr>
<td>antrum.transverse</td>
<td>0.0003 (2.14)</td>
<td>-4.19-4.19</td>
</tr>
<tr>
<td>stomach.antero-posterior</td>
<td>- (0.86)</td>
<td>0-1.59</td>
</tr>
<tr>
<td>stomach.transverse</td>
<td>-0.10 (0.75)</td>
<td>-1.58-1.37</td>
</tr>
<tr>
<td>stomach.longitudinal</td>
<td>0.10 (0.98)</td>
<td>0-2.02*</td>
</tr>
<tr>
<td><strong>Calculations (mm²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACSA</td>
<td>5.8 (30.1)</td>
<td>-53.2-64.8</td>
</tr>
<tr>
<td>spheroid</td>
<td>-0.11 (1.02)</td>
<td>-2.11-1.90</td>
</tr>
<tr>
<td>cylinder + hemisphere</td>
<td>-0.11 (1.59)</td>
<td>-3.23*-3.00*</td>
</tr>
<tr>
<td>cone + hemisphere</td>
<td>-0.12 (0.95)</td>
<td>-1.97-1.74</td>
</tr>
</tbody>
</table>

*Limits of agreement are greater than the relevant differences determined previously ie. 2 mm and 3 mm²

No bias was seen in either the intra- or inter-rater comparisons. Thus, there was no tendency within raters for the first and second measurements to differ systematically, and no evidence of the raters measuring differently (Table 2.4).
Table 2.4  Inter-rater intra-class correlation coefficients (ICC) for the agreement for images measurements for antral cross sectional area (ACSA) and direct stomach raw measurements and calculations presented as intraclass correlations (95% confidence interval)

<table>
<thead>
<tr>
<th>Measurement (mm)</th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>antrum.antero-posterior</td>
<td>0.849 (0.744, 0.908)</td>
</tr>
<tr>
<td>antrum.transverse</td>
<td>0.742 (0.595, 0.835)</td>
</tr>
<tr>
<td>stomach.antero-posterior</td>
<td>0.968 (0.951, 0.979)</td>
</tr>
<tr>
<td>stomach.transverse</td>
<td>0.933 (0.898, 0.956)</td>
</tr>
<tr>
<td>stomach.longitudinal</td>
<td>0.975 (0.958, 0.985)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculations (mm²)</th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSA</td>
<td>0.807 (0.698, 0.877)</td>
</tr>
<tr>
<td>spheroid</td>
<td>0.984 (0.974, 0.989)</td>
</tr>
<tr>
<td>cylinder + hemisphere</td>
<td>0.937 (0.904, 0.959)</td>
</tr>
<tr>
<td>cone + hemisphere</td>
<td>0.891 (0.834, 0.929)</td>
</tr>
</tbody>
</table>

2.6.3 Pre-feed Stomach Residuals

Of the 24 infants with pre-feed stomach images, 18 had measurable gastric residuals, and 6 had completely contracted stomachs. Residual measurements varied between the three calculation methods used, with a median residual volume and range 2.12 mL (0.56–9.27 mL) for spheroid, 2.55 mL (0.65–12.22 mL) for hemisphere + cylinder, and 1.36 mL (0.43–6.70 mL) for hemisphere + cone calculations. The p values for the effect of the calculated feed residual volume on the calculated feed volumes were >0.2 for each method, indicating that knowing and accounting for the stomach residual volumes <9 mL (using spheroid calculation) did not provide a more accurate assessment of the stomach volume. Based on these results, residual volumes were not accounted for in subsequent gastric volume measurements.

2.6.4 Relationship between Stomach Calculations and Delivered Milk Volume

Statistically significant relationships were seen between the delivered feed volume and all stomach volume calculation methods including the ACSA. Individual variations in measurements were observed over time for all techniques (Figure 2.3),
and the direct stomach volume calculation was sensitive to small changes in volume. Accounting for the proportion of the feed already taken and the pre-feed residual, a significant relationship was found between spheroid volume calculations and the known delivered volumes of the same feed (p<0.001) with small discrepancies that were not influenced by feed duration or feed volume.

A significant relationship was seen between hemisphere + cylinder volume calculations and the known delivered feed volumes (p=0.001), however this method had a tendency to under-estimate feed volumes, with the discrepancies increasing as the proportion of feed delivered increased. No association was seen with the pre-feed residual (p=0.86).

A significant relationship was found between the hemisphere + cone volume calculation and the delivered volume (p<0.001), however this method had a tendency to over-estimate feed volumes, with the discrepancies increasing as the proportion of feed delivered increased.

There was a significant relationship between the ACSA and the proportion of feed volume delivered (p <0.001). However, much variation was observed between individual infants. While five (20%) infants demonstrated the expected monotonic increase in ACSA over the duration of the feed a further five (25%) demonstrated the largest ACSA immediately prior to feeding and eight (40%) infants demonstrated the largest ACSA during feed delivery with subsequent reduction as the delivered feed volume increased. Measures of reliability of the ACSA were not performed as for a proportion of individuals the ACSA did not reflect the expected increase in stomach volume that would result from cumulative increases in feed volume during feed delivery (Figure 2.3).
**Figure 2.3** Proportion of maximum calculated volume for stomach as a) spheroid, b) hemisphere + cylinder, and c) hemisphere + cone, and for d) antral cross sectional area (ACSA) measured pre-feed (T0) and at 50% (T50), 75% (T75) and 100% (T100) of feed delivery by intragastric tube.

Differences in ratings of ultrasound image characteristics occurred for 30 of 192 (15.6 %) images. Non-chance agreement (kappa, 95% confidence interval) was highest for echogenicity (0.920, 0.504-0.848, p<0.001) and lowest for curd volume (0.755, 0.606-0.904, p<0.001). Ratings for presence of curd and curd density both achieved a kappa >0.8. These results suggest that inter-rater reliability was very high for ratings of echogenicity, and substantial for ratings of presence of curd, curd density and curd volume.

### 2.6.5 Characterisation of Stomach Echogenicity

All four different classifications of echogenicity were observed in this study (Figure 2.2), with lower echogenicity the least common finding (Table 2.5). Pre-feed (residual) stomach images were frequently more echogenic than the spleen (13/18=72%) and image characteristics changed over the course of the feed (Table 2.5). At completion of the feed, higher echogenicity was seen most often with fortified milk ie. 7/12 (58%) compared to 2/11 (18%) unfortified milk (p=0.006). Lower echogenicity was only associated with unfortified mother’s own milk (p<0.001) while snow storm was more common in unfortified milk (80%, not significant) (Table 2.6).
Table 2.5 Echogenic appearance of breast milk and density and volume of curd during an intra-gastric tube feed

<table>
<thead>
<tr>
<th>Time</th>
<th>More Echogenic</th>
<th>Mixed</th>
<th>Snow Storm</th>
<th>Less Echogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 n=18*</td>
<td>13 (72%)</td>
<td>0</td>
<td>0</td>
<td>5 (28%)</td>
</tr>
<tr>
<td>T50 n=24</td>
<td>8 (33%)</td>
<td>7 (29%)</td>
<td>4 (17%)</td>
<td>5 (21%)</td>
</tr>
<tr>
<td>T75 n=23</td>
<td>8 (35%)</td>
<td>5 (22%)</td>
<td>9 (39%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>T100 n=23</td>
<td>9 (39%)</td>
<td>7 (30%)</td>
<td>5 (22%)</td>
<td>2 (9%)</td>
</tr>
</tbody>
</table>

Density and volume of curd

<table>
<thead>
<tr>
<th>Time</th>
<th>Curd Present</th>
<th>Curd Density</th>
<th>Curd Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 n=18*</td>
<td>16 (89%)</td>
<td>14/16 (88%)</td>
<td>9/16 (56%)</td>
</tr>
<tr>
<td>T50 n=24</td>
<td>17 (71%)</td>
<td>15/17 (88%)</td>
<td>10/17 (59%)</td>
</tr>
<tr>
<td>T75 n=23</td>
<td>17 (74%)</td>
<td>15/17 (88%)</td>
<td>11/17 (65%)</td>
</tr>
<tr>
<td>T100 n=23</td>
<td>19 (83%)</td>
<td>17/19 (89%)</td>
<td>11/19 (58%)</td>
</tr>
</tbody>
</table>

*6 stomachs were completely contracted at T0

Table 2.6 Milk type and echogenicity immediately following feed delivery. Echogenicity is determined relative to the spleen, with ‘mixed’ and ‘snow storm’ ratings having a mixture of brighter and darker sections.

<table>
<thead>
<tr>
<th>Feed Type</th>
<th>n</th>
<th>More Echogenic</th>
<th>Mixed</th>
<th>Snow Storm</th>
<th>Less Echogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfortified MOM</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Fortified MOM</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfortified PDHM</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fortified PDHM</td>
<td>5</td>
<td>4</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

2.7 Discussion

This study found diagnostic ultrasound to be a reliable and accurate method of measuring gastric volume in the preterm infant. While adequate intra- and inter-rater reliability was demonstrated for the ACSA, and for the three direct stomach measurement methods, direct measurement of stomach volume as a spheroid was the most accurate approximation of stomach volume and also allowed for evaluation of stomach contents. This technique is therefore ideal for determining gastric emptying and curding patterns in response to different types of milk feeds as well as tracking
gastric emptying in preterm infants experiencing difficulties in transitioning to full enteral feeds.

Ultrasound is a non-invasive, well tolerated, reproducible, and reliable method of calculating stomach volume over time using the direct measurement of stomach volume (spheroid calculation). Currently there are no published reports of testing for intra and inter-rater reliability for the direct stomach volume calculation or ACSA method in preterm and term infant populations.

Lambrecht et al. (Lambrecht et al., 1988) performed a spheroid calculation of gastric volume with ultrasound although they did not report validation of the measurement technique. We have confirmed that this measurement more consistently estimated stomach volume (Table 2.4, Fig 2.3), suggesting that neither the cone + hemisphere nor hemisphere + cylinder model are appropriate models for preterm infant stomach shape (Table 2.3). The hemisphere + cone model overestimated stomach volumes with the discrepancies increasing as the proportion of feed delivered increased, while the hemisphere + cylinder model consistently underestimated stomach volumes. Further analysis showed that the discrepancies for each of these calculations were not related to feed duration and therefore were not a consequence of gastric emptying during feed delivery.

While we demonstrated a significant relationship between the proportion of feed delivered and ACSA we found that this was the most inconsistent method of tracking gastric emptying (Table 2.4, Fig 2.3). Newell, Chapman & Booth (Newell et al., 1993) previously reported validity of the ACSA method by calculating correlation coefficients for ACSA and volume delivered over the course of an intra-gastric tube feed (n=8); as in our study, a strong linear relationship was demonstrated and significant correlation found (r=0.78, p <0.001). Reproducibility of the ACSA method was determined by comparing a series of ten antral measurements taken over one minute; the method demonstrated reproducibility in a single rater with a mean (SE) coefficient variation of 7.7% (1.1). Small sample size may have prevented the detection of a sizeable proportion of anomalous patterns of change in ACSA over the duration of a feed as we have demonstrated in our study. Further, contraction of the antrum and passage of digesta through the antrum may not have been visualised and recorded during imaging periods of one minute’s duration. The ACSA is the most common
method used in assessing gastric emptying in the preterm infant and it is technically simpler to perform than the direct stomach measurement. Both techniques are relatively quick with minimal disruption to the infant, although some have reported that imaging every 10 minutes may be considered excessive handling for the very preterm infant (Ewer et al., 1994, McClure and Newell, 1996, Newell et al., 1993). Similar to Newell et al. (Newell et al., 1993) we found that measurement of the ACSA is highly repeatable for a single rater, with ICC agreement values >0.95. However, the ACSA was highly variable and less consistent than the direct stomach measurement method. Rather than demonstrating the expected monotonic increase in ACSA over the duration of the feed, 13/24 (54%) infants demonstrated a decrease in ACSA at one point during the observation period (McClure and Newell, 1996, Newell et al., 1993). In addition, Newell et al.(Newell et al., 1993) indicated anomalies in 3/8 (38%) infants; one infant had a reduction in antral area during feeding while two demonstrated no change in antral area from pre-feed to the time point where 25% of the feed volume had been delivered. These anomalous changes may be due to wide fluctuations in ACSA when the antrum is full (Pedersen, 2003), a more rapid transit of feed through the stomach in individual infants, or it may be that the technique requires a plane that is not consistently reproducible or is subject to questionable technique. Differences in infant positioning during scanning, with Newell’s subjects positioned in right lateral position and ours positioned supine, did not impact on the detection of anomalous ACSA findings. Both studies have therefore consistently found individual atypical ACSA responses during feed delivery, suggesting ACSA is not a reliable proxy measure of gastric volume.

Infants routinely had an intra-gastric tube aspirate collected for testing of pH to confirm tube placement as per hospital policy. Although only a minimal aspirate is required for this test, in cases where there is a volume of fluid in the stomach, the sample is easily aspirated and it is clear to the clinician that further fluid is available for aspiration. In our study, in every case only a scant aspirate could be obtained (<0.2 mL) suggesting that the stomach was empty in all of the infants studied. Despite this a measureable pre-feed residual was frequently detected by ultrasound (66%), with some infants having a considerable volume. For example one infant had a calculated residual volume of 9mL that represented 28% of the previous feed (Table 2.5). This is
an important finding particularly in the context of the management of feeding intolerance in the absence of reliable measures of gastric residuals. Given that 26%-48% of neonatal gastric tubes are potentially incorrectly placed (de Boer et al., 2009, Freeman et al., 2012) an ultrasound scan of the stomach would quickly and reliably alert clinicians to the presence of significant gastric residual volume. This is analogous to the routine practice of using ultrasound to calculate urinary bladder residual volumes in patients following prostate or gynaecological surgery (Bodker and Lose, 2003, Palese et al., 2010).

Assessment of the echogenicity of stomach contents showed variable appearances across the duration of intra-gastric feeds of breast milk. Inter-rater reliability for ratings of echogenic appearance, presence and volume of curd were found to be adequate. On completion of feed delivery there was a marked difference in the appearance of fortified (more echogenic, Figure 2.2) and unfortified breast milk (snow storm, Figure 2.2). The type and composition of milk feeds have been shown to impact on gastric emptying in preterm infants (Indrio et al., 2009a, Miller et al., 1990) and may therefore differ in echogenic appearance between different types of milk with regard to volume and density (Khan, 2012b). Indeed Khan (Khan, 2012b) has shown that in term infants, echogenicity of post-feed stomach images is directly related to the casein concentration (p=0.003) and whey:casein ratio (p=0.01) of the milk feed, with the highest casein concentrations associated with mixed echogenicity images. Bovine based human milk fortifier is of a large particle size that is not soluble and has a higher casein content than breast milk and therefore reflects ultrasound waves more than unfortified breast milk. Conversely, the echogenic appearance of unfortified milk indicates a higher aqueous component that may contribute to a more rapid rate of stomach emptying. There are conflicting reports of the effect of human milk fortifiers on gastric emptying rates, with some reporting no difference in emptying (McClure and Newell, 1996, Gathwala et al., 2008) and others reporting faster emptying rates for unfortified breast milk (Ewer and Yu, 1996, Yigit et al., 2008, Ewer et al., 1994). These differences are likely due to the differing compositions of commercial human milk fortifiers. Classification of ultrasound images and their associations with milk type, in conjunction with analysis of gastric emptying patterns,
may prove useful in understanding the mechanisms of gastric emptying patterns in preterm infants.

The direct stomach measurement method of calculating stomach volume offers an accurate, non-invasive technique for monitoring gastric emptying and for detecting and measuring gastric residuals in the preterm population. Use of this technique has the potential to detect and further our understanding of gastric emptying patterns and large stomach residual volumes in the preterm infant. Concomitantly, ultrasound assessment offers further advantages such as the ability to observe the echogenicity and density of stomach contents, and to detect pathologies such as the presence of lactobezoars (Heinz-Erian et al., 2012). Investigation of relationships between gastric emptying and echogenicity of different types of milk feeds will assist in the evaluation and management of preterm enteral nutrition.

2.7.1 Limitations and Implications

These study findings are limited to preterm infants born between 28 and 34 weeks gestation that are fed by intra-gastric tube. Expansion of this study to include serial monitoring of gastric emptying using the direct stomach volume calculation method, and comparison of findings with the published literature would expand our knowledge of normal patterns of gastric curding and emptying in the preterm population over time. The strengths of this study include the number of time points over which gastric volume was measured, and the range of infant ages and feed volumes. The high levels of intra- and inter-rater measurement agreement indicate that the direct stomach volume calculation method has the potential to be adopted as a clinical diagnostic tool.

2.8 Conclusion

Direct ultrasound stomach measurement (spheroid) will provide a useful research tool in investigating gastric emptying in preterm infants. Identification of the presence and nature of residual volumes and intragastric curd may be useful in furthering our understanding of feeding intolerance and gastric emptying patterns in relation to different feed type
Chapter 3 Repeatability of Gastric Volume Measurements and Intragastric Content Using Ultrasound in Preterm Infants

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3.1 Abstract

Objectives: To determine whether serial gastric volumes and intragastric curding are repeatable within individual preterm infants when given sequential feeds of the same volume and composition. Infant and feeding characteristics that might influence differences in measured gastric volumes, echogenicity and intragastric curding were also explored.

Methods: Ultrasound images were used to calculate gastric volumes and to rate echogenicity and intragastric curding for 20 infants. Twenty-nine paired feeds of the same volume and composition were monitored pre and post feed and at 30 minute intervals thereafter. Statistical comparisons of paired gastric volume measurements and agreement between echogenicity and curding ratings were made for each time point. Analyses of factors that influence discrepancies between volume measurements and between curding ratings were performed.

Results: Paired gastric volume measurements were repeatable (ICC=0.971, 0.938 < ICC <0.987). Most (75%) discrepancies were <2mL and increased over time although volume differences were very small. Overall moderate levels of consistency were observed for ratings of echogenicity (κ=0.44), and curd presence (κ≤0.65), density (κ=0.41) and volume (κ=0.47). Gastric emptying during feed delivery is influenced by infant positioning, fortification of human milk, and feeding frequency.
Conclusions: For preterm infants serial gastric volumes are repeatable and ratings of intragastric echogenicity and curding are moderately consistent when fed milk of the same volume and composition. Ultrasound has the potential to further explore factors that influence gastric emptying in the preterm infant.

3.2 Introduction

Normal patterns of gastric emptying and intragastric curding in preterm infants are not well understood. Assessment of gastric emptying has largely been limited to research methods that provide proxy measures of emptying, such as scintigraphy and serial calculations of the antral cross sectional area (ACSA) (Barnett et al., 1999, Ewer et al., 1994). Due to a lack of data for healthy subjects, normal gastric emptying parameters have not been established for preterm infants (Rao et al., 2011). Symptoms of delayed gastric emptying, such as large gastric aspirates, vomiting, and abdominal distension, are related to feeding intolerance and may precede serious complications such as necrotizing enterocolitis (Lin and Stoll, 2006, Lucchini et al., 2011). However assessment of feeding intolerance is based on symptoms rather than objective measures of gastric emptying (Lucchini et al., 2011).

Intragastric curd is thought to influence the rate of gastric emptying, and as curding is dependent on the specific protein composition of milk feeds, may explain in part the previously reported differences in emptying rates between various feed types (Heyman, 1998, Miller et al., 1990). Published data relating to intragastric curding is limited to animal studies and lactobezoar, an associated but rarely diagnosed neonatal complication (Heinz-Erian et al., 2012, Miyazaki et al., 2009, Okada et al., 2010).

Published studies of gastric emptying in preterm infants frequently report an estimate of the minutes since feed delivery at which 50% of the delivered feed volume has emptied from the stomach. This is referred to as the gastric half emptying time and is routinely estimated for gastric emptying study methods including scintigraphy, stable isotope breath tests and applied potential tomography (Bode et al., 2004, Nour et al., 1995, Pozler et al., 2003, van Wijk et al., 2007, Veereman-Wauters et al., 1996), while studies using the ACSA method report 50% change in ACSA as a proxy measure of the gastric half emptying time (Indrio et al., 2009b). Scintigraphy requires exposure to ionizing radiation and as with most other methods, requires the infant to be...
restrained in the supine position for prolonged periods during continuous or serial imaging (Rao et al., 2011). Alternatively ultrasound has been employed to determine gastric emptying patterns in preterm infants using ACSA measurements, and in infants up to 3 months of age using direct stomach measurements from which the stomach volume can be calculated (Ewer et al., 1994, Lambrecht et al., 1988). Recently we have compared these methods in preterm infants and explored alternative algorithms based on observed stomach shapes. A significant relationship was found between the delivered feed volume at set intervals over the course of feed delivery and both the ACSA and direct stomach volume calculations. Stomach volume calculated as a spheroid was more consistently reliable and valid than the alternative calculations based on the assumed shape of the stomach. Further a large proportion of subjects (65%) had at least one sequential ACSA measurement that reduced during feed delivery that was inconsistent with increasing stomach volume (Perrella et al., 2013).

Ultrasound has been employed to assess the effects of different types of human milk fortifiers on gastric half emptying time (Gathwala et al., 2008, Yigit et al., 2008), despite the level of intrindividuallyal variability with regard to gastric emptying not being adequately established in the preterm infant. It is not known whether measures of gastric emptying taken from a single feed are indicative of a consistent pattern for an individual infant. Barnett et al (Barnett et al., 1999) tested reproducibility of the gastric half emptying time by monitoring paired feeds of breastmilk and infant formula using the $^{13}$C octanoic acid breath test. Intraindividual coefficients of variation were reported as 23.9% (Barnett et al., 1999). An interval of up to 5 days between study feeds, and the use of breastmilk feeds that were not controlled for composition are likely to have impacted on these findings. Accurate determination of physiological variability of gastric emptying in the preterm infant requires investigation under strict standardized conditions, controlling for factors that may impact on emptying such as feed composition, feed volume and infant positioning.

There are no established normal parameters for preterm gastric emptying (Lucchini et al., 2011, Kaye, 2011). The reported estimate of gastric half emptying time does not account for differing patterns of stomach emptying such as a lag phase prior
to emptying, nor does it reflect gastric residual volumes. These gaps in knowledge have hindered the use of ultrasound as a clinical tool with which to objectively evaluate feeding intolerance despite current evidence showing it is a repeatable, reliable and accessible method that is more accurate than aspiration to determine pre-feed residual volumes (Perrella et al., 2013).

Furthermore, ultrasound offers the opportunity to investigate intragastric curding of milk feeds and its possible impact on gastric emptying (Heyman, 1998, Khan, 2012a). Intragastric digestion of milk results in the precipitation of casein into curd that appears as a clearly outlined echogenic mass on ultrasound imaging (Miyazaki et al., 2009). Gastric curding and emptying is influenced by the composition of milk feeds. While intragastric curd has been observed in ultrasound examinations of adults and term healthy infants, there are no published studies that explore curd formation in response to milk feeds in term or preterm infants (Cubillos et al., 2012, Khan, 2012a).

Determination of the repeatability of gastric emptying patterns and curding is required to define normal patterns of gastric emptying in stable preterm infants, which would then allow for detection of abnormal patterns that may be associated with preterm gastrointestinal complications.

In order to further assess gastric emptying in the preterm infant while minimizing infant handling, it is important to determine whether a single assessment of gastric emptying is indicative of the typical pattern of gastric emptying and curding.

The aims of the present study were the following:

1. Determine whether the pattern of gastric emptying is repeatable within individual preterm infants when given sequential feeds of the same volume and composition and lying in the same position during and after feeding.

2. Explore infant and feeding characteristics that influence differences in measured stomach volumes between sequential feeds at matched 30 minute time points following feed delivery.

3. Determine whether the echogenicity and curding of stomach contents differ between sequential feeds at matched 30 minute time points following feed delivery.
3.3 Methods

3.3.1 Participants

Medically stable infants born between 28-34 weeks gestation who were receiving full enteral feeds were recruited from the Special Care Nurseries of King Edward Memorial Hospital, Western Australia. Infants with congenital abnormalities, gastrointestinal disease, or symptoms of feeding intolerance within the previous 24 hours were excluded from the study. For multiple birth infants, only one sibling was invited to participate in the study to prevent potential confounding of results.

The Ethics Committees of the Women and Newborn Health Service and The University of Western Australia gave approval for the study. Parents were provided with verbal and written information about the study and provided signed informed consent for their infants’ participation.

3.3.2 Study protocol

Each infant was scanned with ultrasound immediately before and after completion of feed delivery (T0) and at 30 minutes intervals over the course of two consecutive feeds. The 30 minute study intervals, represented as T0 = immediately post feed, T30 = 30 minutes post feed and so on through to T150 = 150 minutes post feed, provided matched pairs of measurements for comparison of stomach volume at the same time points for sequential feeds. Infants were fed either breastmilk or preterm infant formula. Those receiving breastmilk were fed mother’s own milk (MOM) or pasteurized donor human milk (PDHM) from the same batch of milk to ensure identical composition for each of the paired feeds.

Breastmilk feeds included both unfortified and fortified breastmilk. Where human milk fortifier (HMF) was prescribed for study infants, the feeds were fortified with commercially prepared cows milk based HMF. For these infants we studied sequential pairs of both fortified and unfortified feeds; fortification status of the first pair of feeds was determined by using a random numbers. For infants receiving only unfortified milk, a sequential pair of unfortified feeds was studied.

Two different types of HMF were used over the course of the study due to a change in practice in the hospital. S-26 HMF was added at the recommended dose of 1
sachet per 50 mL breastmilk (Wyeth Nutrition, Baulkham Hills, NSW, Australia), and FM 85 HMF was added at the recommended dose of 5 g per 100 mL breastmilk (Nestle Nutrition, Rhodes, NSW, Australia). The biochemical composition differed between the two HMFs (Table 3.1).

### Table 3.1
Biochemical composition and energy content values of S-26 (Wyeth) and FM 85 (Nestlé) human milk fortifiers (HMF) calculated at the prescribed dose for 1L of breastmilk.

<table>
<thead>
<tr>
<th></th>
<th>S-26 HMF</th>
<th>FM 85 HMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein g/L</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Whey g/L</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Casein g/L</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate g/L</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>Lactose g/L</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fat g/L</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Energy kcal/L</td>
<td>140</td>
<td>177</td>
</tr>
</tbody>
</table>

### 3.3.3 Ultrasound Examination

Serial ultrasound stomach images were recorded prior to feeding, immediately following completion of the feed (T0) and every 30 minutes until the next feed was due. All studies were performed using real time two-dimensional ultrasound with the 5PI phased array transducer. To acquire images for calculation of stomach volume, the transducer was positioned perpendicular to the left lateral chest wall and rotated so that the stomach was both at its maximum diameter and length (longitudinal plane), and a clear view of the spleen was achieved. A second image was acquired perpendicular to the longitudinal plane providing a transverse view. The position of the transducer was altered as necessary to compensate for infant movement as per Perrella et al. (Perrella et al., 2013). Scanning duration was <2 minutes at each time point. Images were recorded on the ultrasound machine and transferred to a laptop computer for measurement.
3.3.4 Ultrasound Measurement of Stomach Volume

The direct ultrasound method, whereby measures of longitudinal, transverse and antero-posterior images of the stomach are used to calculate stomach volume based on the assumption that the infant’s stomach is of an ovoid shape, was used. This method has been assessed to have adequate repeatability and reliability (Perrella et al., 2013).

The longitudinal, transverse and antero-posterior measurements were made using ‘Screen Calipers’, V. 4.0 (Iconico Inc. New York, USA). Stomach volume was calculated using the following equation:

\[ \text{Spheroid: longitudinal} \times \text{antero-posterior} \times \text{transverse} \times 0.52 \] (Lambrecht et al., 1988)

The first author selected the images to be measured and conducted image measurements; one each of the transverse and longitudinal planes of the stomach for each time point (10-14 images per infant). Maximum length of the longitudinal stomach image was measured and transverse and anterior-posterior measurements were made of the transverse stomach image. For each infant, all measurements taken from a paired feed were made in a single session. Repeatability and reliability of the first author’s measurements have previously been reported (Perrella et al., 2013)

3.3.5 Echogenicity of Stomach Contents

Data for the sequential preterm formula feeds were excluded from analysis to provide results specific to breastmilk feeds of a consistent composition and volume delivered under similar conditions. Echogenicity of the stomach was assessed by rating the brightness of the longitudinal view of the stomach contents relative to that of the spleen, as described by Perrella et al (Perrella et al., 2013). Briefly, images were classified as having “higher” or “lower” echogenicity than the spleen if this was consistent across the stomach. Areas of higher echogenicity within the stomach were assumed to indicate precipitate or “curd” and were further classified with respect to both density and volume. Curd density was classified as “high”, “medium” or “low” according to whether the echogenic areas appeared to be more echogenic, similar to, or less echogenic than to the spleen. Curd volume was classified as “high” where the echogenic area appeared to occupy >50% of the stomach, and “low” otherwise. Two
classifications of variable echogenicity were used. “Snow storm” referred to images with a predominantly fluid filled stomach (anechoic) with many small bright specks interspersed in the fluid. “Mixed” referred to stomach contents appearing as a combination of lower and higher echogenic areas, indicating a mixture of fluid and semi-solid contents. Images were rated for all feeds at every matched time point.

### 3.3.6 Statistics

#### 3.3.6.1 Sample size determination

The initial study design aimed to recruit 30 infants which were assigned equally into 2 groups of infants fed MOM or PDHM, with two sets of paired feeds (one fortified, one unfortified) to be measured on each infant where possible, for a maximum of n=60 sets of feeds given under identical conditions. Scarcity of infants receiving adequate volumes of PDHM and changes to the fortification regime significantly impacted on recruitment. Use of S-26 HMF was discontinued when n=8 MOM infants had been monitored for fortified feeds. Recruitment ceased during the transition to FM 85 HMF, and recommenced with the aim of recruiting a similar number of feed pairs where the new fortifier had been used. At this point, interim analysis (n=28 feed pairs) indicated we had sufficient data to characterize the differences between sequential feeds, and it was considered unethical to continue recruiting in this fragile population.

#### 3.3.6.2 Statistical analysis

All analyses were performed using R 2.15.2 for Mac OSX (R Development Core Team, 2009) (The R Core Team, 2012). Additional packages nlme (Pinheiro et al., 2008a), irr (Gamer et al., 2007a), and lattice (Sarkar, 2008b) were used for linear mixed modeling, intra-class correlations, and lattice plots respectively. Infant and feed characteristics are presented as median (range) while other values are presented as mean±standard deviation (SD) unless otherwise specified. We considered p-values <0.05 to be significant, and variables were retained in multivariate models only if they were significant at this level; p-values less than 0.001 are reported as p <0.001.

Repeatability of stomach volume measurements was assessed at all time points using a number of statistical methods. Overall agreements between measurements
taken at matched time points for sequential feeds were assessed using two-way intra-class correlation coefficient (ICC) for agreement (two way variance). Intra- and inter-individual variations were quantified using a linear mixed effects model to calculate coefficients of variation using a model that included time as a fixed effect (McGraw and Wong, 1996).

Matched volumes and proportions of delivered feed volumes were compared for immediate post-feed stomach volume measurements (T0) and for final stomach volume measurements (T90 for 2 hourly feeds and T150 for 3 hourly feeds) using Student’s paired samples t-test. Final stomach volume measurements were referred to as “residual” stomach volumes and analyses of these included only the final stomach volumes of the study feeds to ensure consistency of feed composition and volume. Multivariable analysis was conducted to investigate simultaneous effects and interactions of factors such as feed volume and feed frequency on T0 volume using linear mixed effects modeling with stomach volume as the response, feed frequency and time as predictors and infants as groups.

Linear mixed effects modeling was used to determine influences on differences in paired stomach volume measurements for all time points. Due to heteroskedasticity of the residuals for the absolute value data, a square root transformation was used prior to linear mixed effects modeling of this data. Absolute values were used to determine the effects of predictors on the magnitude of the volume differences between paired measurements.

Ratings of echogenicity and intragastric curd were compared between sequential breastmilk feed pairs (1 formula feed pair excluded from analysis) by calculating Cohen’s Kappa (κ) statistic to determine the level of non-chance agreement between ratings, and confidence intervals were assessed. Adequacy of agreement was assessed as modest if $0.7 < \kappa < 0.8$, and adequate if $\kappa \leq 0.8$ (Nunnally and Bernstein, 1994a). Logistic regression analysis was performed to determine whether agreement between paired ratings of echogenicity and intragastric curd was influenced by factors such as feed frequency, fortification, feed time as a continuous variable or as a factor, feed volume, or the difference in feed durations.
3.4 Results

3.4.1 Infant Characteristics

A total of 20 infants were recruited with data for 30 pairs of sequential feeds (MOM: n=24; PDHM: n=3, PTF n=1; Table 3.2). Data was complete for 21 of 30 paired feeds. Missing data occurred when the infant required medical or nursing care at the time of the scheduled scan (T120: n=3, T150; n=9), when feed delivery was prolonged (n=1) causing an overlap between the scheduled scan and delivery of the next feed (T150; n=1). For one infant on CPAP the images were unsuitable for measurement for one feed. Four of the paired feeds were found not to meet the study inclusion criteria: 3 infants required repositioning from the supine to prone position due to increased desaturation episodes after delivery of the second paired feed, and one infant developed symptoms of feeding intolerance within 24 hours of the study feeds. Data for these feeds were retained for analysis on an intention-to-treat basis.

Table 3.2 Infant and feed characteristics for 2 hourly and 3 hourly sequential feed pairs: milk type, human milk fortifier (HMF) type, feed volume in milliliters (mL) and milliliters per kilogram (mL/kg), feed duration, infant corrected gestational age, postnatal age and weight.

<table>
<thead>
<tr>
<th>Feed type and fortification</th>
<th>2 hourly feeds</th>
<th>3 hourly feeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfortified breast milk</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Breastmilk + S-26 HMF</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Breastmilk + FM 85 HMF</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Preterm infant formula</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>

**Feed characteristics median (range)**

<table>
<thead>
<tr>
<th></th>
<th>2 hourly feeds</th>
<th>3 hourly feeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed volume (mL)</td>
<td>19 (17-25)</td>
<td>33 (30 – 55)</td>
</tr>
<tr>
<td>Prescribed feed (mL/kg)</td>
<td>13 (10-14)</td>
<td>19 (18-21)</td>
</tr>
<tr>
<td>Feed duration (min)</td>
<td>12 (6-18)</td>
<td>15 (8-40)</td>
</tr>
</tbody>
</table>

**Infant characteristics median (range)**

<table>
<thead>
<tr>
<th></th>
<th>2 hourly feeds</th>
<th>3 hourly feeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected age (weeks)</td>
<td>33^{16} (31^{14} - 34^{13})</td>
<td>33^{12} (31^{11} – 34^{16})</td>
</tr>
<tr>
<td>Postnatal age (days)</td>
<td>14 (10-22)</td>
<td>17 (8 – 35)</td>
</tr>
<tr>
<td>Weight (grams)</td>
<td>1565 (1300 – 1835)</td>
<td>1907 (1550 – 2865)</td>
</tr>
</tbody>
</table>
Infants (11 female, 8 male) included in the analysis were median (range): birth gestation 31+3 weeks (28-32+6); birth weight 1600 g (910-2370); corrected gestational age 33+3 weeks (31+1–34+6); study weight 1718 g (1300–2865) and postnatal age 16 days (8-35). Two infants were small for gestational age (birth weight <10th centile) and one was large for gestational age (birth weight >90th centile). The median (range) age at commencement of full enteral feeds was 9 days (5–13); study feed volume 30 mL (17-55) and feed mL/kg 18 mL/kg (10–21), and feed duration 15 (6–40) minutes. The majority (26/30) of study feed pairs were commenced in the morning. Two infants received nasal CPAP, and one received phototherapy. Prescribed medications (including caffeine, vitamin D, ferrous sulfate, nystatin and probiotics) were administered to study infants at the prescribed times: none of these are known to affect gastric emptying.

3.4.2 Repeatability of Post Feed Stomach Volume Measurements

Gastric volumes measured at matched time points for sequential feeds were found to be very similar, regardless of the pattern of gastric emptying (Figure 3.1). There was a high level of agreement between stomach volume measures at matched time points (ICC=0.971, 0.938<ICC<0.987); a higher level of agreement and confidence intervals were obtained when pre-feed residuals and measurements for the infant with feed intolerance were excluded (ICC=0.984, 0.976<ICC<0.989).

Both intra- and inter-individual variation increased over time, although differences in actual volume measurements were smaller at later time points. Intra-individual variation ranged from 14.3% at T0 to 49.3% at T90 and 60.9% at T150, while inter-individual variation ranged from 32.7% at T0 to 53% at T90 and 136.1% at T150.

Stomach volume discrepancies >3 mL were observed in 24/170 (14.1%) measurements, with 10/170 (5.9%) being ≥ 5 mL. Exclusion of data for the feed intolerant infant, and of pre-feed residual stomach volumes made minimal difference to the results; discrepancies >3 mL were observed in 20/140 (14.3%) with 6/140 (4.2%) discrepancies >5 mL. Discrepancies >3 mL were observed across all time points but were most prevalent for final stomach volume measurements (8/24, 33%) and in the first 30 minutes after feed delivery (11/24, 46%).
Figure 3.1 Gastric emptying curves for preterm infants fed sequential feeds of a) breastmilk fortified with FM 85 human milk fortifier, b) unfortified breastmilk and c) breastmilk fortified with S-26 human milk fortifier.
3.4.3 Repeatability of Immediate Post Feed Stomach Volumes (T0)

There was a high level of agreement between paired T0 stomach volumes with minimal discrepancies of <1 mL (range 0.08–4.24mL) in more than half of the 28 paired feeds. A strong positive relationship was found between the delivered feed volume and post-feed stomach volume (p<0.001). As previously outlined, 4 of 28 paired feeds did not meet the study inclusion criteria for the full duration of the study period. Intention to treat analysis including data from these infants did not impact on the significance of the relationship between delivered volume and post-feed stomach volume were similar to results based on 24 paired feeds that met all study inclusion criteria (p=0.001).
Immediately post-feed, neither stomach volumes (p=0.50) nor proportion of feed remaining in the stomach (p=0.29) differed between the paired feeds. For both 2 hourly and 3 hourly feeds, the paired stomach volume measurements were not significantly different (2 hourly: p=0.13; 3 hourly p=0.83). Proportions of delivered feed volume were not significantly different for 3 hourly feeds (p=0.72) and a borderline trend was observed for 2 hourly feeds (p=0.09). Significantly higher proportions of delivered feed volume were retained at T0 for 2 hourly feeds (mean±s.e. 94.6%±6.8%) than for 3 hourly feeds (74.3%±6.2%, p=0.041).

3.4.4 Repeatability of Residual Stomach Volumes

Final residual stomach volumes were measured at 90 minutes post feed for 2 hourly feeds and at 150 minutes post feed for 3 hourly feeds; this generally occurred 10-15 minutes prior to commencement of the next feed. A contracted stomach (volume=0 mL) was observed in 9/28 (32%) for 2 hourly feeds and 14/22 (64%) at T150 for 3 hourly feeds.

The measured final stomach volumes and proportions of delivered feed volume were mean (range) 3 mL (0–12.3 mL) and 14% (0%-49%) for 2 hourly feeds, and 0.9 mL (0–3.8 mL) and 2.8% (0%-13%) for 3 hourly feeds. Mean differences between paired stomach volumes (2 hourly: p=0.34; 3 hourly: p=0.79) and between paired proportions of delivered feed volumes (2 hourly: p=0.33; 3 hourly: p=0.64) were not significant.

3.4.5 Influences on Repeatability of Immediate Post Feed Stomach Volumes (T0)

No relationship was seen between the T0 volume and residual stomach volume of the previous feed after taking into account the feed volume, either as a main effect (p=0.82) or as an interaction with the feed volume (p=0.28). Thus pre-feed residual volumes were excluded from the analysis of post-feed stomach volume measurements.

The T0 stomach volume was consistently found to measure less than the delivered feed volume. For an average feed volume of 30 mL the average T0 stomach volume was 23 mL. Larger feed volumes were associated with larger T0 stomach volumes, with an average additional 0.9 mL of stomach volume for each additional 1 mL of feed volume delivered (p<0.001).
After accounting for feed volume, it was found that smaller T0 stomach volumes were associated with prone and right lateral positioning, and longer feed duration, while larger T0 stomach volumes were associated with fortification with FM 85 HMF and 2 hourly feed frequency. Infants positioned prone during feeding had significantly smaller T0 stomach volumes (p=0.013) while a borderline trend was observed for those positioned right lateral (p=0.074) compared to supine positioning. For each additional minute of delivery time, the T0 stomach volume was on average 0.2mL lower (p=0.039). The effect of the duration of feed delivery was not different for feeds of different volumes (p=0.46).

T0 stomach volumes for feeds fortified with FM 85 were on average 3.6 mL more than for feeds of the same average volume of both unfortified breastmilk and that fortified with S-26 HMF (p=0.004). T0 stomach volumes were proportionately larger for 2 hourly feeds than for 3 hourly feeds after accounting for fortification, infant position, feed volume and feed duration (p=0.035).

3.4.6 Influences on Repeatability of Stomach Volumes

Larger discrepancies between paired stomach volumes were strongly associated with differences in feed duration with a feed of longer duration resulting in smaller stomach volume (p=0.002), differences in echogenicity ratings (p=0.028), and time as a linear trend with larger volume discrepancies seen at T120 and T150 (p=0.001). While statistically significant, the magnitudes of the effects were very small. For differences in duration of feed delivery the discrepancy was 0.4 mL per 30 minutes post feed while for differences in echogenicity ratings and time post feed, the discrepancies were 0.2 mL and -0.1 mL per 30 minutes post feed respectively.

3.4.7 Repeatability and Patterns of Echogenicity of Stomach Ultrasound Images

The overall level of agreement for echogenicity ratings was 60.3% (κ=0.44, Table 3.4) with higher κ ratings observed for unfortified (κ=0.51) than for fortified breastmilk feeds (S-26 HMF κ=0.36, FM 85 HMF κ=0.38, Table 3.3). Discrepancies between echogenicity ratings for paired feeds occurred across all time points and were similar between 2 hourly (37%) and 3 hourly (42%) feeds. The majority of pre-feed discrepancies (9/13=69%) were accounted for by the occurrence of one contracted
stomach in paired images. Agreements between echogenicity ratings were not associated with feed frequency, milk fortification status or type, feed volume, or differences in feed duration.
Table 3.3 Agreement between ratings of echogenicity and curd at all time points for sequential feeds of unfortified breastmilk and breastmilk fortified with human milk fortifier (HMF) as assessed by Kappa and 95% Confidence Intervals (95% CI).

<table>
<thead>
<tr>
<th>Feed type</th>
<th>n</th>
<th>Curd present*</th>
<th>Kappa (95% CI)</th>
<th>Curd Volume</th>
<th>Kappa (95% CI)</th>
<th>Curd Density</th>
<th>Kappa (95% CI)</th>
<th>Echogenicity</th>
<th>Kappa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfortified breastmilk</td>
<td>57</td>
<td>95%</td>
<td>0.77 (0.52, 1.0)</td>
<td>68%</td>
<td>0.48 (0.27, 0.69)</td>
<td>75%</td>
<td>0.47 (0.24, 0.71)</td>
<td>36%</td>
<td>0.51 (0.35, 0.68)</td>
</tr>
<tr>
<td>Breastmilk + S-26 HMF</td>
<td>50</td>
<td>96%</td>
<td>0.48 (0.0, 1.0)</td>
<td>72%</td>
<td>0.40 (0.15, 0.65)</td>
<td>64%</td>
<td>0.31 (0.05, 0.56)</td>
<td>58%</td>
<td>0.36 (0.17, 0.56)</td>
</tr>
<tr>
<td>Breastmilk + FM 85 HMF</td>
<td>34</td>
<td>100%</td>
<td>1.00 (1.0, 1.0)</td>
<td>71%</td>
<td>0.46 (0.16, 0.75)</td>
<td>68%</td>
<td>0.34 (0.04, 0.64)</td>
<td>59%</td>
<td>0.38 (0.13, 0.63)</td>
</tr>
</tbody>
</table>

*Curd was always present when there was a measurable stomach volume. Disagreement only occurred if one of two matched stomach images showed a contracted stomach.
Table 3.4 Agreement between ratings of echogenicity and curd at each matched time point for sequential feeds of unfortified and fortified breastmilk as assessed by Kappa and 95% Confidence Intervals (95% CI).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Curd present*</th>
<th>Curd Volume</th>
<th>Curd Density</th>
<th>Echogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kappa (95% CI)</td>
<td>% agreement</td>
<td>Kappa (95% CI)</td>
</tr>
<tr>
<td>Overall</td>
<td>171</td>
<td>97% (0.49, 0.82)</td>
<td>70% (0.37, 0.60)</td>
<td>70% (0.28, 0.53)</td>
<td>60% (0.34, 0.55)</td>
</tr>
<tr>
<td>T0</td>
<td>30</td>
<td>100% Yes = 100%</td>
<td>63% (0.58)</td>
<td>53% (0.47)</td>
<td>67% (0.48)</td>
</tr>
<tr>
<td>T30</td>
<td>30</td>
<td>100% Yes = 100%</td>
<td>60% (0.54)</td>
<td>70% (0.72)</td>
<td>57% (0.32)</td>
</tr>
<tr>
<td>T60</td>
<td>30</td>
<td>100% Yes = 100%</td>
<td>73% (0.72)</td>
<td>73% (0.75)</td>
<td>57% (0.29)</td>
</tr>
<tr>
<td>T90</td>
<td>30</td>
<td>93% (0.45, 1.0)</td>
<td>80% (0.32, 0.89)</td>
<td>67% (0.12, 0.71)</td>
<td>53% (0.04, 0.58)</td>
</tr>
<tr>
<td>T120</td>
<td>14</td>
<td>93% (0.23, 0.32)</td>
<td>79% (0.13, 0.99)</td>
<td>79% (0.05, 0.92)</td>
<td>79% (0.32, 0.99)</td>
</tr>
<tr>
<td>T150</td>
<td>7</td>
<td>71% (0.0, 1.0)</td>
<td>71% (0.0, 1.0)</td>
<td>71% (0.0, 1.0)</td>
<td>57% (0.0, 0.78)</td>
</tr>
</tbody>
</table>

*Curd was always present when there was a measurable stomach volume. Disagreement only occurred if there was one contracted stomach within two matched stomach images.
Across all feeds, ratings of lower echogenicity and snow storm were not observed beyond T60, while mixed and higher were identified across all time points (Table 3.4). Ratings of lower were infrequent; the highest incidence was at T30 (13/58=22%). Overall ratings of snow storm were 28/58 (48%) with the most prevalent at T0. Images of contracted stomachs identified by the echogenic juxtaposed anterior and posterior stomach walls indicated no measurable stomach volume; for these images echogenicity and curding ratings were recorded as contracted.

Patterns of echogenicity differed between 2 hourly and 3 hourly feeds. The most prevalent ratings for 3 hourly feeds were T0=snow storm; T30=mixed; T60=mixed; T90=higher; T120=higher; T150=contracted, while for 2 hourly feeds mixed was the most prevalent rating across all time points.

3.4.8 Repeatability and Patterns of Curd Presence, Density and Volume

Curd was present whenever there was a measurable stomach volume, and thus disagreements occurred only when the stomach was contracted for one of a pair of stomach images. As no contracted stomachs were identified in the hour after feeding, there was absolute (100%) agreement between paired ratings at T0, T30 and T60. Overall agreement was 96.5% (κ=0.65, Table 3.4). Curd rating agreement and Kappa are reported in Table 3.3 for the different milk types, and in Table 3.4 for the matched time points.

The proportions of overall agreement for curd density ratings were 75% (κ=0.47) for unfortified feeds, 64% (κ=0.31) for feeds with S-26 HMF and 68% (κ=0.34) for feeds with FM 85 HMF (Table 3.3). Discrepancies and ratings of curd density were similar between 2 hourly and 3 hourly feeds. High curd density was the most prevalent rating; low (14-30%) and medium (4-14%) ratings were less common across all time points.

Overall agreement between curd volume ratings was 70.2% (κ=0.47), and was similar between fortified and unfortified milk feeds (68-72%, Tables 3.2 and 3.3). The level of non-chance agreement was low in the first hour post feed (κ=0.19 – 0.38) and across all subsequent time points (κ=0.42–0.61, Table 3.4). Ratings of high curd volume typically increased as the stomach emptied.
### 3.5 Discussion

Our analyses of paired stomach volume measurements at matched time points indicate that when fed milk of the same volume and composition under similar conditions, stable preterm infants’ serial gastric volume measurements are repeatable. Statistical comparison of paired measures at multiple time points rather than a single estimated gastric half emptying time has provided more extensive information regarding gastric emptying rates over time than previously published. Of the paired stomach volumes measured, most (75%) were discrepant by less than 2mL, with an intraindividual coefficient of variation (CV) of 14.2% immediately after the feed. These results indicate a high level of repeatability between sequential feeds of both MOM and PDHM when fed under similar conditions (Figure 3.1). Differences in paired stomach volumes increased with time, in that lower stomach volumes were associated with proportionately greater differences. Gastric residual volumes were common, with a greater proportion of empty stomachs identified in infants receiving 3 hourly feeds when compared to those receiving 2 hourly feeds (64% vs. 32% respectively).

Reports of repeatability of gastric emptying in the preterm population are limited. Barnett et al (Barnett et al., 1999) used the $^{13}$C octanoic acid breath test to determine repeatability of gastric emptying in healthy preterm infants and reported an intraindividual CV of 23.9% for gastric half emptying time. Wide individual variations were reported including discrepancies between compared gastric half emptying times of >30 minutes in 4 infants. Results are likely to have been confounded by an interval of up to 5 days between study feeds of the same volume, over which time changes in maturation and health status may have influenced results. Further, 71% of the study infants were fed breastmilk that was not controlled for macronutrient composition therefore it is highly likely that the comparison feeds were of variable composition. The known large intraindividual variability of macronutrients such as fat and protein in breastmilk may potentially impact gastric emptying (Ballard and Morrow, 2013, Kent et al., 2006, Siegel et al., 1985). Pozler (Pozler et al., 2003) used the $^{13}$C octanoic acid breath test to determine gastric half emptying times in response to increasing feed volumes in 16 preterm infants during establishment of enteral feeds of pasteurized donor human milk. Study feeds were monitored when feed volumes reached 7, 7-13
and 10-19mL/kg of birth weight; infants were born between 31 – 37 weeks gestation, were 11-38 hours of age at commencement of the study and 63-227 hours of age at completion. The reported intraindividual mean CV of 11.5% for gastric half emptying time is similar to our findings of an overall CV of 14.2%, but applies to a population of newborn preterm infants establishing enteral feeding, with measures likely to have been influenced by incongruent infant positioning and feed volumes of variable composition, and possibly changes in maturation and health status over time. Despite these differences it is interesting that the variability is similar over time and with varying volumes to that of sequential feeds of the same volume and composition. It would be valuable to explore this further to determine whether the rate or pattern of gastric emptying changes as the preterm infant matures.

Post feed (T0) stomach volumes were consistently smaller than delivered feed volumes, with feeds of longer duration associated with a greater reduction in post feed stomach volumes. These findings reflect the initiation of gastric emptying during feed delivery and are consistent with emptying of liquid feeds, in contrast to the lag phase that has been observed with emptying of solid meals (Lin et al., 2000, Neu, 2007). Factors that influenced gastric emptying during feed delivery included: feed frequency, fortification and infant positioning. Less gastric emptying occurred during feed delivery of 2 hourly feeds when compared to 3 hourly feeds. Gestational and postnatal ages were similar between infants fed 2 hourly and 3 hourly suggesting that other characteristics such as feed volume or feed mL/kg may also influence emptying during feeding (Table 3.2). Larger feed volumes have been associated with faster gastric emptying in adults and children (Fruehauf et al., 2009, Schmitz et al., 2012). Emptying during bolus feed delivery has not been previously described and further investigation may reveal whether early gastric emptying differs in infants with feeding intolerance.

Due to changes in nutrition policy we were able to opportunistically compare the gastric emptying of two different HMFs. We found that the stomach volumes immediately post feed that reflect emptying during milk delivery, were similar between unfortified breastmilk and milk fortified with S-26 but significantly higher for feeds fortified with FM 85 (p = 0.041). However, fortification with FM 85 did not significantly influence stomach volumes at subsequent time points. While casein is
associated with curd formation, we found that fortification with S-26, a HMF containing casein, did not impact on gastric emptying while fortification with FM 85, a HMF with a protein component consisting entirely of hydrolyzed whey, was associated with a larger gastric residual volume post feed. Secretion of GIP peaks in the first 15-20 minutes of gastric emptying, increasing gastric secretions and the rate of passage of stomach contents to the duodenum (Calbet and Holst, 2004, Edholm et al., 2010). Plasma concentrations of GIP are significantly higher following ingestion of a whey protein hydrolysate solution when compared to ingestion of solutions containing intact whey, intact casein and casein peptide hydrolysate, and gastric secretion volumes are 50% higher in the first hour (Calbet and Holst, 2004). There is some evidence that hydrolyzed whey proteins are relatively slowly digested \textit{in vivo} by the duodenal enzymes of preterm infants (Lindberg et al., 1998). We propose that initial rapid delivery and detection of hydrolyzed whey proteins to the preterm duodenum may cause early initiation of the ileal brake with feedback causing slowed gastric emptying around the time of feed delivery, and with increased gastric secretions contributing to the larger post-feed stomach volume. Falling GIP serum concentrations subsequent to its peak at 15-20 minutes from the commencement of feeding would allow for an increased rate emptying.

The observed slower emptying during feeding of milk fortified with FM 85 contrasts with Ewer & Victor (Ewer and Yu, 1996) who reported a mean gastric half emptying time for milk fortified with FM 85 that was more than double that of unfortified breastmilk (48 min versus 21 min). Both studies are limited by their small samples sizes (this study FM 85; n=7; Ewer & Victor n=11) however Ewer & Victor used the ACSA method which is much more variable than the method we have employed and may have contributed to the discrepant findings. The technique we have used more accurately assesses gastric volume differences between fortified and unfortified milk as well as differences between fortifiers and it has the potential for clinicians to utilize this method to assess changes in the feeding management of unwell infants or policy changes in nutrition.

It is commonly believed that positioning of the infant influences the rate of gastric emptying. We found that infants lying prone had significantly lower post feed
stomach volumes (T0), with a trend to lower stomach volumes for right lateral positioning with the largest stomach volumes were observed in those positioned supine. While post-feed stomach volumes were significantly lower in infants positioned prone when compared to those positioned supine, the effects of prone positioning were not significant at subsequent time points. This is incongruent with a number of studies that report prone positioning results in smaller stomach volumes at various time points after feed delivery, although none have assessed post feed stomach volumes. Yu used a dye dilution technique and Cohen used direct stomach aspiration to measure and compare gastric residual volumes for different infant positions and found significantly lower gastric volumes were at 30 or 60 minutes post feed for infants positioned prone and right lateral compared to supine positioning (Yu, 1975, Cohen et al., 2004). Limited measurements were made, with gastric volumes measured at 30 minutes (Yu, 1975) or 60 and 180 minutes post feed (Cohen et al., 2004). Hwang et. al (2003) and Chen et. al (2013) each performed measurements of gastric aspirates at 30 minute intervals commencing 30 minutes after the feed. Hwang reported significantly smaller stomach volumes at 30 minutes post feed for infants positioned prone when compared to those positioned left lateral, while Chen reported that infants positioned prone had significantly lower stomach volumes at every time point from 30 to 150 minutes compared to those positioned supine. The aspiration technique does not accurately measure infant stomach volume as only the fluid portion and not curd can be measured (Perrella et al., 2013) thus overestimating the rate of emptying. It seems that the effect of body positioning on preterm gastric emptying is more complex than currently thought and requires further studies that include precise serial assessments of gastric volume between feeds to more accurately describe body position effects. This is important, as body positioning is often one of the first strategies used to encourage gastric emptying in preterm infants with signs of feeding intolerance and gastro-oesophageal reflux (Omari et al., 2004).

Unlike dye dilution methods, the ultrasound method of measuring stomach volumes to monitor gastric emptying allows for quantification of emptying during feeding without disrupting physiological processes (Ruhl et al., 1995). It also avoids overestimation of gastric emptying that may potentially occur in methods such as
ACSA, scintigraphy and aspiration techniques that typically consider the immediate post feed measurement to reflect the maximum gastric volume with no correction for emptying during feeding. Typically post feed values are standardized to 100% and subsequent measures are reported as a proportion of this value rather than the actual radiographic area measurement, dye dose or feed volume delivered (Ewer et al., 1994, Hwang et al., 2003, Lin et al., 2000). Knowledge of the delivered feed volume and the volume after delivery of the feed enables more accurate assessment of gastric emptying both during and subsequent to feeding.

This is the first study to investigate the factors influencing gastric emptying of sequential feeds by comparing discrepancies in gastric volume at multiple time points between the feeds. Volume discrepancies were more prevalent as time since feed delivery increased, although the actual volume differences were small. Whilst statistically significant differences in duration of feed delivery and echogenicity ratings were associated with volume discrepancies, the volume differences were very small (0.2mL more and 0.1mL less per 30 minutes post feed respectively). Further, when we compared measures of repeatability between our ‘intention to treat’ data set and that of infants that met all study criteria for the duration of the study, they were surprisingly similar. These findings suggest that when feed volume and composition are controlled for, reliability of gastric emptying in preterm infants is robust with regard to external influences such as duration of feed delivery and curding.

The echogenicity of the stomach contents somewhat reflects digestion of the milk in the stomach with snow storm and lower echogenic ratings (associated with higher fluid volumes and very low curd) more prevalent in the first 30 minutes after feed delivery, followed by mixed and higher echogenicity ratings indicating higher curd volume and less fluid. We found that when there were differences in stomach volumes at each time point there were also differences in echogenicity or curding. It is possible that the formation of semi-solid intragastric curd slows gastric emptying in preterm infants (Heyman, 1998, Lin et al., 2000) although the differences in stomach volumes observed for discrepant echogenicity ratings were extremely small (≤1 mL). The curding process in preterm infants has not been thoroughly explored with respect to stomach acidity, gestational and corrected age, and milk composition. Further
simultaneous investigation of intragastric curding and gastric emptying that accounts for infant maturation and milk composition may help to more precisely determine influences on curding and emptying in preterm infants.

In this study the appearance of the stomach contents suggest that after the first 90 minutes post feed most of the fluid portion (whey) of the feed had passed through the stomach. Subsequent reductions in intragastric curd volumes were observed with two thirds of the infants having an empty stomach two and a half hours after the feed, for 3 hourly feeds. The sequence was different for 2 hourly feeds with the predominant rating across all time points being mixed and only a third of infants had an empty stomach at an hour and a half after the feed. The infants fed 3 hourly were not different to those fed 2 hourly with respect to corrected gestational age and postnatal age (Table 3.2); therefore discrepant curding sequences are unlikely to be related to reduced gastric acid production associated with either earlier gestational or postnatal ages (Armand et al., 1996). Compared to those fed 3 hourly, infants fed 2 hourly had lower body weights and lower feed volumes, both as mL/kg and as total feed volume delivered (Table 3.2). While a larger liquid meal volume is associated with faster gastric emptying in adults, one study of preterm infants suggests that feed volumes do not influence emptying in the newborn period (Kwiatek et al., 2009, Pozler et al., 2003). The effect of volume on emptying and curding has not been clearly established in the healthy preterm population and warrants further investigation.

While intragastric curd is a common feature of stomach images for both fortified and unfortified breastmilk feeds, the sequence of echogenicity and curd ratings had low levels of consistency between consecutive feeds of the same composition and volume (Table 3.4). Unfortified milk tended to be more consistent than fortified breastmilk feeds (Table 3.3). For two infants, differences in echogenicity can be explained by large differences in feed duration, but for the remainder, variability of echogenicity and curding is difficult to explain where sequential feeds have been matched for composition, volume and adjusted for feed duration. Logistic regression analyses did not identify any significant factors associated with discrepancies in both echogenicity and curd ratings. Differences in stomach volume were ≤1 mL and so are unlikely to explain the differences. While preterm infants’
gastric acid and pepsin secretions in response to feeds of breastmilk and preterm formula are not reported to be different (Henderson et al., 2001), intragastric proteolysis of breastmilk and skim cow’s milk differs widely in both preterm and term infants (Berfenstam et al., 1955). It seems reasonable to expect similar responses to breastmilk fortified with bovine based HMF although this has yet to be established. Also, it is not known whether secretion of gastric acid and enzymes is consistent between feeds and whether this might explain the variability and echogenicity of curding between sequential feeds.

### 3.6 Conclusion

Patterns of gastric emptying are repeatable for preterm infants when fed milk of the same volume and composition under similar conditions. In addition a portion of the feed is emptied from the stomach during feed delivery. Depending on the HMF used, fortified milk may empty more slowly than unfortified milk during feed delivery. Two hourly feeds empty more slowly during feeding, resulting in higher post feed stomach volumes that are not related to either infant gestation or postnatal age compared to three hourly feeds. Ultrasound has the potential to further explore factors that influence digestion and gastric emptying in the preterm infant.
Chapter 4 Influences on breastmilk composition on gastric emptying of preterm infants

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4.1 Abstract

Objectives: To determine whether specific biochemical and energy concentrations influence gastric emptying of unfortified and fortified mother’s own milk (MOM) in stable preterm infants, and whether gastric emptying differs between feeds of unfortified MOM and feeds fortified with S-26 or FM 85 human milk fortifier (HMF) when fed the same volume under similar conditions. Influences of infant gestation, age and weight, and feed characteristics were also explored.

Methods: Stomach volumes of 25 paired unfortified and fortified MOM feeds were monitored pre and post feed delivery and at 30 minute intervals thereafter. For each feed MOM samples were analysed to determine concentrations of total protein, casein, whey, carbohydrate, lactose, fat, and energy. Fortified feed compositions were calculated by adding fortifier biochemical and energy concentrations to unfortified MOM concentrations. Ultrasound images were used to calculate infant stomach volumes. Statistical comparisons were made of paired stomach volume measurements.

Results: Higher feed concentrations of casein were associated with faster gastric emptying during feed delivery (p=0.007). When compared to unfortified MOM, S-26 fortified feeds emptied similarly while FM 85 fortified feeds emptied more slowly both during feed delivery and over the postprandial period (p=0.002, p<0.001). Gastric emptying was slower for 2 hourly feeds compared to 3 hourly feeds (p=0.003) and in
supine position compared to prone ($p=0.001$).

**Conclusions:** Breastmilk composition influences gastric emptying in stable preterm infants, with feeds of higher casein concentration emptying faster during feeding than otherwise equivalent feeds, and FM 85 fortified MOM emptying more slowly than unfortified MOM.

### 4.2 Introduction

Mother’s own milk (MOM) fortified with a multicomponent human milk fortifier (HMF) is recommended for preterm infants born <1500 g to achieve satisfactory weight gain and neurodevelopment (2012). While preterm formula offers the advantage of faster short-term growth, it is associated with an increased risk of necrotizing enterocolitis and late onset sepsis (Quigley and McGuire, 2014, Schanler et al., 2005) and so is generally only used in the absence of human milk. Feeding tolerance and the attainment of full enteral feeding is enhanced by human milk (American Academy of Pediatrics, 2012) although the mechanism for this is poorly understood, primarily due to a limited understanding of how milk composition influences preterm gastrointestinal motility.

Gastric emptying in preterm infants is reported to be mature from 34 weeks gestation (Riezzo et al., 2009). Enterogastric neural reflexes as well as a nutrient and osmotic receptors trigger an inhibitory feedback system that regulate the rate of gastric emptying to ensure adequate time for digestion and absorption of macronutrients (Pearson et al., 2013, Van Citters and Lin, 2006). Hydrolytic end products of digestion of fat, carbohydrate and protein stimulate the release of enterogastrones such as GLP-1 and PYY thus slowing gastric emptying through initiation of the ileal brake, with fatty acids noted to have the most powerful effect in adults (Van Citters and Lin, 2006, Hellstrom et al., 2006, Shin et al., 2013).

Breastmilk has been shown to empty from the stomach more rapidly than formula (Van Den Driessche et al., 1999, Ewer et al., 1994, Indrio et al., 2009b). The effects of differing compositions of infant formulae have been studied, with faster emptying observed in those where the predominant fat is medium-chain triglyceride compared to long-chain triglyceride; where carbohydrate consists of glucose polymers
compared to glucose and lactose; and where protein is extensively hydrolyzed whey protein compared to partially hydrolyzed whey, intact casein or intact whey proteins (Siegel et al., 1985, Staelens et al., 2008). While breastmilk is the recommended source of nutrition for infants, there are no published studies examining the effect of different breastmilk biochemical compositions or human milk fortifiers on gastric emptying in preterm infants.

Breastmilk composition varies widely both within and between women, particularly with regard to fat and protein (Ballard and Morrow, 2013, Kent et al., 2006). The variability of breastmilk with respect to its biochemical and caloric content suggests that gastric emptying may vary both between and within infants according to the composition of individual feeds, particularly after fortification.

Fortification of milk for preterm infants is virtually universal, however evidence for the effect of HMF on gastric emptying is limited and inconclusive (Ewer and Yu, 1996, Gathwala et al., 2008, McClure and Newell, 1996, Yigit et al., 2008). Methodological issues such as small numbers of serial gastric emptying measurements, the use of study feeds where breastmilk composition was not controlled for, and differences in HMF compositions between studies may have contributed to the incongruous findings. With limited published evidence and the wide variations in breastmilk and HMF composition, it is largely unclear whether fortification impacts gastric emptying in the preterm infant.

Knowledge of the composition of each feed is crucial to enable exploration of the biochemical factors that may contribute to intra- and inter-individual differences in gastric emptying between feeds of unfortified and fortified MOM. Therefore, in this study we aimed to:

1. Investigate whether specific biochemical compositions and energy concentrations influence gastric emptying of unfortified and fortified MOM;
2. Determine whether gastric emptying differs between feeds of unfortified MOM and feeds of MOM fortified with S-26 HMF or FM 85 HMF, when infants are fed the same volume under similar conditions;
3. Explore infant and feed characteristics that influence gastric emptying of unfortified and fortified MOM.
4.3 Methods

4.3.1 Participants

Medically stable infants between 28-34 weeks gestation receiving full enteral feeds of MOM were recruited from the Special Care Nurseries of King Edward Memorial Hospital, Western Australia. Infants with congenital abnormalities, gastrointestinal disease, or symptoms of feeding intolerance within the previous 24 hours were excluded. For multiple births, only one sibling was recruited to prevent potential confounding of results.

The Ethics Committees of the Women and Newborn Health Service and The University of Western Australia gave approval for the study. Parents were provided with verbal and written study information and provided signed informed consent.

4.3.2 Study protocol

Paired unfortified and fortified MOM feeds were studied for each infant, with the order of fortification status randomized and inter-feed interval < 72 hours. Attending clinicians prescribed the feed volume, frequency and positioning of each infant. The same infant position (supine, right lateral, or prone) was maintained for each of the paired feeds during feed delivery and postprandially. Study feeds coincided with scheduled feed times.

Ultrasound scans were performed immediately post feed delivery (T0), at 30 minutes after completion of feed delivery (T30), and at 30 minute intervals thereafter until the next feed was due ie. T90 for 2 hourly feeds and T150 for 3 hourly feeds. Sterile scan gel (PDI, New York) was pre-warmed in the infants’ incubators, or used at room temperature for infants in cots.

Intragastric tube feeds were delivered by gravity, with pre-feed gastric aspirates tested for acidity to confirm correct tube placement. Feeds were given at room temperature. All infants maintained their body temperature within normal limits and tolerated the study procedure well.

Two HMFs were used over the course of the study due to a change in practice in the study setting. S-26 HMF was added at the recommended dose of 1 sachet per 50 mL breastmilk (Wyeth Nutrition, Baulkham Hills, NSW, Australia), and FM 85 HMF was
added at the recommended dose of 5g per 100mL breastmilk (Nestlé Nutrition, Rhodes, NSW, Australia). The protein content of the two HMFs differed; S-26 HMF contained partially hydrolyzed casein and whey proteins at a reported ratio of 40:60 while FM 85 contained 100% extensively hydrolyzed whey protein.

4.3.3 Analysis of feed composition

Unfortified MOM samples (3 to 5 mL) were collected immediately prior to the addition of HMF on the morning of the study. Samples were stored in sterile polypropylene capped tubes (Sarstedt, Nümbrecht, Germany) at 4°C until delivery of the study feed, and then frozen at -20°C for later biochemical analysis. One milk sample was collected when consecutive unfortified and fortified MOM feeds were prepared from the same pool of MOM. When studies of fortified and unfortified MOM occurred on separate days, milk samples were collected on each day.

Milk samples were analyzed to determine total protein (g/L), casein, whey, lactose and fat, and the results were used to calculate energy concentration as calories per 30mL (cal/30mL) using standard laboratory assay methods as described by Khan et al (Khan et al., 2013). Biochemical analysis of MOM feeds containing HMF was not possible as our laboratory assays have not been validated for partially and extensively hydrolyzed proteins, and manufacturers do not disclose commercial HMF composition details. The absence of information regarding the specific mixture of amino acids and peptide bonds in HMF precludes the development of suitable protein assays for fortified breastmilk. Therefore the total biochemical and energy concentrations of fortified feeds were calculated by adding the composition data provided by the HMF manufacturers to the measured unfortified MOM concentrations.

4.3.4 Ultrasound examination and calculation of stomach volume

Real time two-dimensional ultrasound was performed using portable ultrasound (Sonologic SonoScape S6, Australia) with a 5PI phased array transducer. To acquire images for calculation of stomach volume, the transducer was positioned perpendicular to the left lateral chest wall and rotated so that the stomach was both at its maximum diameter and length (longitudinal plane), and a clear view of the spleen was achieved. A transverse image was acquired perpendicular to the longitudinal
plane. Scanning duration was <3 minutes at each time point. Images were recorded on the ultrasound machine and transferred to a laptop computer for measurement.

Image measurements of the longitudinal, transverse and antero-posterior axes were made using ‘Screen Calipers’, V. 4.0 (Iconico Inc. New York, USA) by the first author, who was blinded to details other than the infants’ study identification numbers and study dates. Stomach volume was calculated using the following equation, which has been assessed to have adequate repeatability and reliability (Perrella et al., 2013):

\[
\text{Spheroid: longitudinal axis } \times \text{ antero-posterior axis } \times \text{ transverse axis } \times 0.52
\]

(Lambrecht et al., 1988)

4.3.5 Statistical analysis

The initial study design required recruitment of 30 infants to receive paired feeds (one fortified MOM, one unfortified MOM) under identical conditions within paired feeds. A change in nutrition policy resulted in discontinuation of S-26 HMF when 12 infants had been monitored for paired fortified and unfortified feeds. Recruitment ceased during the transition to FM 85 HMF, and recommenced with the aim of recruiting a similar number of feed pairs where the new fortifier had been used. Interim analysis of a previous preterm gastric emptying study indicated that 14 paired feeds provide an adequate sample to detect significant differences in gastric volume measurements and so recruitment ceased when 14 unfortified / FM 85 HMF fortified pairs had been studied (Perrella et al., 2014).

All analyses were performed using R 2.15.2 for Mac OSX (R Development Core Team, 2009). Additional packages nlme (Pinheiro et al., 2008a) and lattice (Sarkar, 2008b) were used for linear mixed modeling, and graphical exploration of the data, respectively. Descriptive statistics are presented as median (range) for infant and feed characteristics, and as mean and standard deviation (SD) for all other measures, unless otherwise specified. We considered \( p \) values <0.05 to be significant, and values less than 0.001 reported as <0.001. Predictors were retained in multivariate models only if they were significant at the \( p <0.05 \) level, and borderline results (0.05 <\( p <0.1 \)) are discussed.
Both measured stomach volumes and proportions of delivered feed volume (% feed volume) were analyzed to provide both specific individual measures of emptying during feeding and comparisons between feeds of different volumes. The effect of fortification on stomach volumes and % feed volumes were examined using linear mixed effects modelling with milk fortification status and time as predictors, and individual infants as the grouping variable. Variables for the initial model were determined by identifying all predictors that were significant after accounting for time and fortification status. Differences in pre-fortification composition between the 10 feeds that were not studied sequentially were accounted for in the multivariable model.

To test for non-linear effects of post-feed time, two formulations were considered, being time as a $2^\text{nd}$ polynomial, and time as a categorical variable. As the 2 hourly feeds did not have the same number of measurements as the 3 hourly feeds, the quadratic model was chosen. This was retained as the default in all models, testing the curve and trend separately.

Starting from a model where all univariately significant predictors were included, the final model was selected by sequentially omitting non-significant variables until all remaining fixed-effects variables had marginal $p$ values <0.05, and then testing for significance of each of the omitted variables when added to this model. Where additional significant variables were found, this process was repeated. In the situation where two covariates were significant when added to the current model individually, but neither were significant when both were included, both were retained in the model to account for their effects.

Considered covariates for influences on measured stomach volumes and calculated proportions of feed delivered were: birth gestation, birth weight, appropriateness of weight for gestational age, gender, postnatal age, corrected gestational age, infant weight, feed volume, feed frequency, feed millilitres per kilogram (mL/Kg), pacifier use during feeding, infant body position, use of continuous positive airway pressure (CPAP), feed energy value (cal/30mL), and the following biochemical concentrations measured as total grams per litre (g/L): protein, casein,
whey, carbohydrate, lactose and fat. Appropriateness of the models was assessed with visual inspection of residual plots.

Differences with respect to fortification status in frequencies of empty stomachs, residuals >3mL, and residuals >30% of delivered feed volume were tested for using Fisher's Exact Test; these were performed separately for the two feed frequencies. Influences on residual stomach volumes were assessed using linear mixed effects modelling with milk fortification status, feed volume and feed duration as predictors and individual infants as the grouping variable.

4.4 Results

4.4.1 Infant characteristics

Of the 26 infants recruited; one infant received one feed of S-26 fortified PDHM due to insufficient MOM and so was excluded from the analysis, leaving data for 25 pairs of fortified and unfortified MOM feeds (S-26 HMF: n = 11; FM 85 HMF: n = 14). Milk composition data was not available for 3 feeds (1 unfortified, 2 S-26 fortified) from 2 infants leaving 23 pairs of feeds for analysis. Missing data occurred when infants required care at the time of the scheduled scan (T120; n = 1, T150; n = 5).

Infants (14 female, 11 male) included in the analysis were mean±SD (range): birth gestation 30.1±1.4 weeks (28-32.9), birth weight 1331±275 g (910-1910), corrected gestational age 33.1±1.3 weeks (31.4–36), and postnatal age 21±9 days (10-42). Six infants were small for gestational age (SGA; birth weight <10th centile) with the remainder appropriately grown. Age at commencement of full enteral feeds was 10±4 days (6-20); delivered feed volume 27±9.4 mL (14-42), feed mL/kg 16±3.2 mL/kg (12–22), and feed duration 13±4 min (7-20). Infants receiving 2 hourly feeds were younger and smaller than those receiving 3 hourly feeds (Table 4.1). Two infants received nasal CPAP. Prescribed medications including caffeine, vitamin D, ferrous sulfate, nystatin and probiotics were administered to study infants at the prescribed times: none of these are known to affect gastric emptying. Infants did not receive the same medications at each of the paired study feeds. As the combined volume of administered medications was typically ≤1mL it is unlikely that this would influence differences in gastric volume measurements.
Table 4.1 Feed tallies for mother’s own milk (MOM) and fortified MOM feed pairs by feed frequency and human milk fortifier (HMF) type, and infant and feed characteristics reported as mean±SD (range).

<table>
<thead>
<tr>
<th>Feed pair characteristics</th>
<th>2 hourly feeds</th>
<th>3 hourly feeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOM±S-26 HMF</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>MOM±FM 85 HMF</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Total pairs</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Feed volume (mL)</td>
<td>19±3.3 (14–25)</td>
<td>35±4.5 (30–42)</td>
</tr>
<tr>
<td>Prescribed feed (mL/kg)</td>
<td>13±1 (12-15)</td>
<td>19±1.8 (18–22)</td>
</tr>
<tr>
<td>Feed duration (min)</td>
<td>12±3.2 (7–18)</td>
<td>15±6.6 (8–20)</td>
</tr>
</tbody>
</table>

Infant characteristics

<table>
<thead>
<tr>
<th>Infant characteristics</th>
<th>2 hourly feeds</th>
<th>3 hourly feeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected age (weeks)</td>
<td>32.6±1 (31.4–34.6)</td>
<td>33.6±1.1 (31.4–36)</td>
</tr>
<tr>
<td>Postnatal age (days)</td>
<td>18±6 (11–35)</td>
<td>23±10 (10–42)</td>
</tr>
<tr>
<td>Weight (grams)</td>
<td>1404±238 (1090–1835)</td>
<td>1790±225 (1550–2250)</td>
</tr>
</tbody>
</table>

4.4.2 Feed composition

Total biochemical and energy concentrations are reported in Table 4.2 with significant differences observed between fortified and unfortified MOM for mean carbohydrate, protein, whey, casein and energy concentrations.
Table 4.2 Biochemical composition (g/L) and energy content (cal/30mL) values of S-26 and FM 85 human milk fortifiers (HMF) at the prescribed dose for 1L of milk, and of unfortified mother’s own milk (MOM) and MOM fortified with S-26 HMF and FM 85 HMF reported as mean±SD (range).

<table>
<thead>
<tr>
<th></th>
<th>S-26 HMF</th>
<th>FM 85 HMF</th>
<th>MOM</th>
<th>MOM + S-26 HMF</th>
<th>MOM + FM 85 HMF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=23</td>
<td>n=6</td>
<td>n=17</td>
<td>n=17</td>
<td>n=17</td>
</tr>
<tr>
<td>Protein</td>
<td>10</td>
<td>10</td>
<td>19.4±4.8</td>
<td>28.4±3.8***</td>
<td>29.5±3.8***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12.6–29.0)</td>
<td>(24.3–37.1)</td>
<td>(24.5–39.1)</td>
</tr>
<tr>
<td>Whey</td>
<td>6</td>
<td>10</td>
<td>15.0±4.1</td>
<td>19.5±3.4*</td>
<td>24.6±3.8***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(9.2–24.0)</td>
<td>(13.3–26.2)</td>
<td>(19.2–29.6)</td>
</tr>
<tr>
<td>Casein</td>
<td>4</td>
<td>0</td>
<td>3.2±3.7</td>
<td>9.1±4.6***</td>
<td>2.2±1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.6–13.9)</td>
<td>(5.3–16.5)</td>
<td>(0.7–5.7)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>24</td>
<td>35</td>
<td>54.1±5.2</td>
<td>84.2±10.5***</td>
<td>89.0±5.4***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(46.6–60.3)</td>
<td>(71.2–112.4)</td>
<td>(81.1–94.1)</td>
</tr>
<tr>
<td>Lactose</td>
<td>0</td>
<td>0</td>
<td>54.1±5.2</td>
<td>60.2±10.5</td>
<td>54.0±5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(46.6–60.3)</td>
<td>(47.2–88.4)</td>
<td>(46.1–59.1)</td>
</tr>
<tr>
<td>Fat</td>
<td>2</td>
<td>0.2</td>
<td>39.1±15.9</td>
<td>35.5±13.2</td>
<td>40.8±7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(17.5–60.6)</td>
<td>(20.5–47.4)</td>
<td>(28–60.8)</td>
</tr>
<tr>
<td>Energy</td>
<td>4.2</td>
<td>5.3</td>
<td>18.8±4.3</td>
<td>22.4±4.1*</td>
<td>24.1±2.2***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12.0–24.7)</td>
<td>(16.4–28.7)</td>
<td>(21.4–29.9)</td>
</tr>
</tbody>
</table>

* p<0.01, *** p<0.001
4.4.3 Influences on gastric emptying during feeding

Immediately post feed, the retained feed proportion differed by the total casein concentration of the feed and by milk fortification status. For each additional 1g/L of casein the retained feed proportion was 2.6% lower (p=0.007). Compared to unfortified MOM, FM 85 fortified feeds had retained feed proportions on average 11% higher (p=0.006) and stomach volumes 2.8 mL higher (p=0.002) while S-26 fortified feeds had stomach volumes 3.6mL lower (p=0.014, Table 4.3).

The T0 stomach volume consistently measured less than the delivered feed volume. For an average feed volume of 30 mL the average T0 volume was 21 mL. Larger feed volumes were associated with larger T0 stomach volumes, with each additional 1mL of delivered feed volume resulting in an additional 1.8 mL at T0 (p<0.001).

T0 stomach volumes and retained feed proportions were influenced by feed frequency as well as postnatal age, feed mL/kg and CPAP. Compared to 3 hourly feeds, the retained feed proportion was on average 13.4% higher for 2 hourly feeds (p<0.001). After accounting for feed volume, small but significant effects of postnatal age and feed mL/kg were observed. Stomach volumes were 1.3 mL lower for each additional postnatal week (p=0.031) with a trend towards lower % feed volumes (p=0.065), and were 1 mL lower for each 1 mL increase in feed mL/kg (p=0.03). For the two infants receiving CPAP, retained feed proportions were 36% higher (p<0.001) than those not receiving respiratory support.

4.4.4 Influences on postprandial gastric emptying

S-26 fortified feeds emptied similarly (p=0.23) to unfortified MOM while retained feed proportions of FM 85 fortified feeds were on average 5.6% higher (p<0.001) across the postprandial period. Further, 2 hourly feeds of FM 85 fortified MOM had retained feed proportions on average 8.3% higher than that fed 3 hourly (p=0.002). While feeds of unfortified MOM and of S-26 fortified MOM had curvilinear patterns of gastric emptying with the rate of emptying slowing over time, feeds of FM 85 fortified MOM emptied at a more consistent rate that approximated a linear
pattern of emptying (p=0.046, Figure 4.1). Stomach volumes decreased over time, with the emptying rate decreasing as the stomach emptied (p<0.001).

Infant positioning and feed frequency had large influences on gastric emptying throughout the postprandial period, while infant weight and feed volume had small but significant effects. Infants positioned supine had retained feed proportions on average 11% higher than those in prone or right lateral positions (p<0.001) while two hourly feeds had retained feed proportions on average 10.4% higher than that of 3 hourly feeds (p=0.012). For each 100 g increase in infant weight the retained feed proportion was 2.5% higher (p<0.001), and larger feed volumes were associated with higher retained feed proportions over time (p<0.001). There was a non-significant trend towards faster postprandial emptying in SGA infants with retained feed proportions on average 6% lower across the postprandial period (p=0.053).

In constructing the multivariable model for postprandial gastric emptying, individually there were significant effects of infant weight and feed mL/kg, but on including both in the model, one cancelled out the effect of the other, suggesting a relationship between the variables. This is explained by the use of infant weight in the calculation of feed mL/kg.
Figure 4.1 Gastric emptying curves for preterm infants fed paired feeds of unfortified mother’s own milk (MOM) and MOM fortified with a) S-26 human milk fortifier or b) FM 85 human milk fortifier
**Table 4.3** Significance (p-values) of selected predictors for measured stomach volumes (volume) and calculated proportions of delivered feed volume (% feed) immediately post feed (T0) and for subsequent serial post feed time points. Variables were included in the table if they were composition variables, or if they were significant in at least one model.

Bold text indicates p-values for the significant variables retained in the final model. All other p-values come from models containing the final model plus the specified predictor. Coefficients are presented only for variables in the final model. For categorical variables, these indicate the average difference between categories. For continuous variables, these indicate the average change per unit increase in the predictor.

<table>
<thead>
<tr>
<th>T0 post feed</th>
<th>Serial post feed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume (mL)</strong></td>
<td><strong>% Feed</strong></td>
</tr>
<tr>
<td>coefficient</td>
<td>p</td>
</tr>
<tr>
<td>Feed fortification</td>
<td></td>
</tr>
<tr>
<td>MOM + FM 85</td>
<td>2.8</td>
</tr>
<tr>
<td>MOM + S-26</td>
<td>-3.6</td>
</tr>
<tr>
<td>Feed Composition g/L</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.36</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.66</td>
</tr>
<tr>
<td>Fat</td>
<td>0.23</td>
</tr>
<tr>
<td>Protein</td>
<td>0.74</td>
</tr>
<tr>
<td>Whey</td>
<td>0.29</td>
</tr>
<tr>
<td>Casein</td>
<td>0.43</td>
</tr>
<tr>
<td>cal/30mL</td>
<td>0.37</td>
</tr>
<tr>
<td>Covariates</td>
<td></td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>-1.3</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>0.59</td>
</tr>
<tr>
<td>Infant position</td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>7.0</td>
</tr>
<tr>
<td>Right lateral</td>
<td>0.39</td>
</tr>
<tr>
<td>Nasal CPAP</td>
<td>0.60</td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td>Quadratic</td>
<td>NA</td>
</tr>
<tr>
<td>Linear</td>
<td>NA</td>
</tr>
<tr>
<td>Feed volume</td>
<td>1.8</td>
</tr>
<tr>
<td>Feed freq</td>
<td></td>
</tr>
<tr>
<td>3 hourly</td>
<td>13.4</td>
</tr>
<tr>
<td>2 hourly</td>
<td>-1.0</td>
</tr>
<tr>
<td>Included interactions</td>
<td></td>
</tr>
<tr>
<td>FM 85 x 2 hourly feeds</td>
<td>-12.6</td>
</tr>
<tr>
<td>2 hourly feeds x time</td>
<td>NA</td>
</tr>
</tbody>
</table>

* linear term not considered here, as the quadratic term was significant. 1 Gestational age (days), birth weight (g), gender, corrected gestational age (weeks), pacifier use, feed duration (min) did not significantly add to the model (p>0.1) and have been omitted from this table. Small for gestational age was marginal (p=0.053) and so also omitted from the table.
**Gastric residual volumes**

Empty stomachs were less frequent for 2 hourly feeds (29% at T90, n=24) than 3 hourly feeds (62% at T150, n=21, Table 4.4). For 3 hourly feeds, empty stomachs were more prevalent for unfortified feeds (83%, n=12) than fortified feeds (FM 85 HMF: 43%, n=7; S-26 HMF: 0%, n=2); assuming that missing data for fortified feeds were for empty stomachs, the incidence of empty stomachs 56% (n=9) and 50% (n=4) respectively would still be considerably lower than for unfortified MOM.

Higher residual volumes were associated with larger feed volumes \( (p=0.043) \) and shorter feed delivery \( (p=0.058) \) for 2 hourly feeds but not for 3 hourly feeds. After accounting for these in 2 hourly feeds, S-26 fortified feed residuals were similar to those of unfortified feeds \( (p=0.179) \), and FM 85 fortified feed residuals were significantly higher (average 2 mL higher, \( p=0.015 \), Table 4.4). Similarly, FM 85 fortified feed residuals were significantly higher (average 1.1 mL higher, \( p=0.040 \)) for 3 hourly feeds. Residual stomach volumes >3 mL were observed in 46% of 2 hourly feeds and were infrequent following 3 hourly feeds (Table 4.4).

One gastric residual volume >30% feed volume was observed following a fortified 2 hourly feed (Table 4.4). Accounting for feed duration, the effect of fortifier on the retained % feed volume was significant, with FM 85 fortified feeds on average 9.9% higher than for unfortified feeds of the same duration \( (p=0.018) \), although the effect of feed duration was not significant \( (p=0.076) \), and the effect was small (~1% for each additional minute of feed duration). Feed volume was not associated with gastric residuals as % feed volume remaining in the stomach.
Table 4.4 Gastric residual volumes for mother’s own milk (MOM) feeds reported for all feeds, unfortified MOM, MOM fortified with S-26 human milk fortifier (HMF) and MOM fortified with FM 85 HMF. Counts and proportions of empty stomachs (stomach volume = 0 mL), average residual stomach volumes reported as mL mean ±standard deviation, stomach volumes >3mL and counts and proportions of delivered feed volume (% feed) >30% are reported for 2 hourly feeds at 90 min post feed and 3 hourly feeds at 150 min post feed.

<table>
<thead>
<tr>
<th></th>
<th>All feeds</th>
<th>MOM</th>
<th>MOM + S-26 HMF</th>
<th>MOM + FM 85 HMF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 hourly feeds</strong></td>
<td>n=24</td>
<td>n=12</td>
<td>n=4</td>
<td>n=8</td>
</tr>
<tr>
<td>Empty stomach</td>
<td>7 (29%)</td>
<td>4 (33%)</td>
<td>1 (25%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Residual volume</td>
<td>2.4±2.2</td>
<td>1.7±1.6</td>
<td>2.2 ±1.8</td>
<td>3.6±2.2*</td>
</tr>
<tr>
<td>Volume &gt;3 mL</td>
<td>11 (46%)</td>
<td>5 (42%)</td>
<td>1 (25%)</td>
<td>5 (63%)</td>
</tr>
<tr>
<td>% feed &gt;30%</td>
<td>1 (4%)</td>
<td>0</td>
<td>0</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td></td>
<td>n=21</td>
<td>n=12</td>
<td>n=2</td>
<td>n=7</td>
</tr>
<tr>
<td>Empty stomach</td>
<td>13 (62%)</td>
<td>10 (83%)</td>
<td>0</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>Residual volume</td>
<td>1.0±1.5</td>
<td>0.4±1.0</td>
<td>1.7±0.1</td>
<td>1.9±1.5*</td>
</tr>
<tr>
<td>Volume &gt;3 mL</td>
<td>2 (9%)</td>
<td>0</td>
<td>0</td>
<td>2 (29%)</td>
</tr>
<tr>
<td>% feed &gt;30%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* p = 0.015

4.5 Discussion

This study has shown that higher casein concentrations in MOM feeds facilitated gastric emptying during delivery of the feed to preterm infants. The differing compositions of FM 85 and S-26 HMF influenced gastric emptying in that FM 85 fortified feeds emptied more slowly than unfortified feeds both during feed delivery and across the postprandial period, while S-26 fortified feeds emptied faster during feed delivery (Table 4.3). The differences are likely explained by stimulation of the ileal brake, which alters according to the dose and chemical structure of the various end products of digestion (Van Citters and Lin, 2006). While statistically significant, the magnitude of the effects of MOM casein concentration and HMFs on gastric emptying are small and not of clinical concern for the stable preterm infant.

MOM feeds with higher total casein concentrations emptied faster during feed delivery. Each additional 1g/L of casein resulted in a 2.6% lower feed proportion immediately post feed. The effect was not sustained over the postprandial period and so is unlikely to influence feeding tolerance in the preterm infant. While it has been
assumed that casein predominant infant formula slows gastric emptying, the evidence does not support this (Woodley and Mousa, 2008). Extensive intragastric hydrolysis of casein facilitates faster gastric transit, with casein solutions emptying faster than whey solutions in adults during the immediate post feed period (Hall et al., 2003). Subsequent slowing of gastric emptying is likely explained by the increased nutrient load delivered to the intestine and/or detection of other triggers of the ileal brake.

Similarly feeds of MOM fortified with S-26 HMF, that has a whey:casein ratio of 60:40, had post-feed stomach volumes on average 3.6 mL less than unfortified feeds. However the lack of effect on postprandial gastric emptying suggests that these associations are not clinically significant. Typically gastric emptying of fortified MOM is slower or similar to unfortified milk (Ewer and Yu, 1996, Gathwala et al., 2008, McClure and Newell, 1996, Yigit et al., 2008). Differences in casein content and properties between HMFs may account for these findings.

Due to differences in physicochemical structures, human milk caseins are more extensively hydrolyzed in the stomach than bovine caseins (Miller et al., 1990, Armaforte et al., 2010). The physical structure of hydrolyzed proteins in HMF is not known, however the partial hydrolysis of HMF bovine caseins could potentially result in structures comparable to human milk caseins. This would explain the similarity in gastric emptying rates for S-26 fortified MOM and unfortified feeds. Further, caseins are more extensively hydrolyzed than whey proteins resulting in rapid emptying of high casein feeds during feed delivery without triggering the release of enterogastrones (Henderson et al., 2001). Subsequently the increased nutrient load delivered to the intestine and/or detection of other triggers of the ileal brake would reduce gastric emptying to a rate similar to unfortified feeds.

The gastric transit of FM 85 fortified feeds was slower than unfortified feeds both during feed delivery and postprandially. This agrees with the literature (Ewer and Yu, 1996) where the gastric half emptying time for FM 85 fortified milk was significantly slower (48 min vs 21 min) and had a more linear pattern than unfortified milk. While we found statistically higher retained feed proportions at each time point no complications were observed in our stable preterm subjects although it is not possible to predict the impact on feeding intolerance in susceptible infants (Ofek
Slower emptying of FM 85 fortified feeds may be due to the HMF protein content that consists solely of extensively hydrolyzed whey protein, which increases GIP secretion (Calbet and Holst, 2004, Edholm et al., 2010). In turn GIP is associated with increased gastric secretions and delayed gastric emptying thus explaining in part our results.

Osmolality of the feeds may have also played a role in the slower gastric transit of FM 85 fortified MOM (Kreissl et al., 2013). Hyperosmolar feeds stimulate duodenal osmoreceptors that slow gastric emptying and increase gastric secretions (Pearson et al., 2013). While measurement of osmolarity was outside the scope of this study, the osmolar range for unfortified preterm breastmilk is 263 to 370 mOsmol/L (Thatrimontrichai and Janjindamai, 2009, Yigit et al., 2008). FM 85 fortified preterm breastmilk has a reported osmolarity of 356 mOsmol/L (Nestlé Nutrition, Rhodes, NSW, Australia) however osmolarity of fortified breastmilk increases over time (De Curtis et al., 1999) with reported values as high as 472 mOsm/L (95% CI 428–545 mOsm/L) for FM 85 fortified thawed milk (Kreissl et al., 2013). As the recommended maximum osmolarity for infant feeds is 400 mOsmol/L (Pearson et al., 2013), it is possible that the addition of HMF caused a hyperosmolar load resulting in slowed gastric emptying.

Feed energy concentrations did not influence gastric emptying most likely due to the relatively small differences between unfortified and fortified feeds so the ileal brake was not stimulated. Further the evidence is conflicting as to whether energy concentrations do impact gastric emptying in preterm infants, with feeds in the range of 5 to 20 cal/30mL having no effect and higher concentrations of 20 and 24 cal/30mL showing higher retained feed proportions at 80 minutes post feed (Siegel et al., 1985, Ramirez et al., 2006).

Both feed volume and infant positioning influenced gastric emptying, although the observations are based on inter-individual differences. Larger 3 hourly feeds emptied more quickly than smaller 2 hourly feeds. This is consistent with evidence from adult and paediatric studies that higher feed volumes empty more rapidly (Fruehauf et al., 2009, Schmitz et al., 2012), with scant data available for preterm infants (Ramirez et al., 2006). Accounting for feed volume, infants positioned prone
had faster gastric emptying than those positioned supine during both feed delivery and the postprandial period. Again whilst the prone position is thought to facilitate gastric emptying evidence is not consistent. Two of four published studies report no difference in serial postprandial gastric residual volumes between supine and prone positions (Chen et al., 2013, Cohen et al., 2004, Hwang et al., 2003, Yu, 1975), suggesting the need for more robust investigations.

Increasing postnatal age and feed mL/kg were associated with lower immediate post feed stomach volumes, while increasing infant weight was associated with higher postprandial retained feed proportions, although the magnitudes of the effects were very small. There was also a trend towards faster emptying during feeding for SGA infants. While this is of clinical interest, the number of SGA infants was insufficient to determine whether this is a true association.

Final residual volumes are routinely measured via aspiration and volumes >3mL together with other markers may prompt closer monitoring for feeding intolerance (Lucchini et al., 2011). Mean gastric residual volumes in this study were typically <2.5 mL as expected for the stable preterm infant. While FM 85 fortified feed residual volumes were statistically significantly higher than those of unfortified feeds, the actual volume difference was very small and therefore not considered problematic (Table 4.4). Empty stomachs were more common for 3 hourly feeds (2 hourly: 29%; 3 hourly: 62%) and for unfortified MOM (2 hourly: 33%; 3 hourly: 83%). Feeding frequency and volume influence gastric residuals therefore it is likely that one-third to two-thirds of infants may have an empty stomach and cue for a feed before the scheduled feed time. In these cases consideration should be given to flexible feeding times as cue based feeding is associated with a more rapid transition to full oral feeding (Kirk et al., 2007).

In the absence of a fluid pre-feed gastric aspirate it is usually assumed that the healthy tube fed preterm infant has an empty stomach prior to feeding. However, we have shown with ultrasound that residual curd is often present (Perrella et al., 2014). It is possible that the presence of stomach contents is advantageous for infants considering the healthy human fetus’ stomach is never empty, and early enteral feeding rather than fasting results in better feeding tolerance (Sase et al., 2000, SIFT
Investigators Group, 2013). The presence of a small gastric residual enables continuous gastric emptying of breastmilk feeds, which may contribute to the development of gastrointestinal function and the intestinal microbiome.

4.6 Conclusion

Higher casein concentrations in MOM feeds is associated with faster gastric emptying during feed delivery, but not in the subsequent postprandial period. The biochemical composition of HMF influences gastric emptying where postprandial emptying of S-26 fortified MOM is similar to unfortified MOM, while FM 85 fortified MOM empties more slowly both during feeding and in the postprandial period. The size of these effects, while statistically significant are not of clinical concern, making it unlikely that either MOM milk composition or fortification with S-26 or FM 85 HMF contribute to feeding intolerance in the stable preterm infant.
Chapter 5 Gastric emptying and curding of pasteurized donor human milk and mother’s own milk in preterm infants.

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5.1 Abstract

**Background:** To determine whether gastric emptying and curding of unfortified and fortified pasteurized donor human milk (PDHM) differ from that of mother’s own milk (MOM) in preterm infants. Influences of feed composition, infant and feed characteristics were also explored.

**Methods:** Paired feeds of unfortified and fortified PDHM (n=15) and MOM (n=17) were studied in preterm infants born <34 weeks gestation. Gastric volumes and curding were determined from ultrasound images, with feeds monitored immediately post feed and at 30 minute intervals thereafter. Feed compositions were calculated from laboratory measures of unfortified milk (protein, casein, whey, lactose, fat, total energy) and fortifier composition data as provided by the manufacturer. Linear mixed effects models were used to determine the influence of milk type and composition on serial gastric volume measurements, and gastric curding described.

**Results:** Immediate post feed and subsequent postprandial retained feed proportions were higher for PDHM (23%, p=0.026; 15%, p=0.006) respectively, and for fortified feeds (31.5%; 8.8%, both p<0.001). Higher casein, whey and lactose concentrations were associated with lower immediate post feed proportions (all p<0.001), and higher casein concentrations had lower subsequent postprandial feed proportions (p<0.006). Curding of PDHM was similar to that of MOM.

**Conclusion:** Pasteurization and fortification of breastmilk slow gastric emptying. While statistically significant, the magnitude of the differences in retained feed proportions are small and unlikely to be of clinical concern for the stable preterm infant.
5.2 Introduction

Currently mother’s own milk (MOM) is fortified to improve the nutrition of very low birth weight infants (2012, Arslanoglu et al., 2013, World Health Organization and UNICEF, 2003). Following preterm birth, many mothers experience delayed secretory activation and may be unable to provide enough breastmilk to meet their infants requirements (Hill et al., 2005). In the absence of MOM, pasteurized donor human milk (PDHM) is the preferred alternative to preterm formula (2012, World Health Organization and UNICEF, 2003).

The rate of gastric emptying is an important component of feeding tolerance in preterm infants, with signs of delayed emptying such as large gastric residual volumes, vomiting and a distended abdomen prompting the interruption of enteral feeds (Moore and Wilson, 2011). Gastric emptying is influenced by the composition of the feed with MOM emptying faster than formula (Cavell, 1981, Ewer et al., 1994), and formula with medium chain triacylglycerol emptying faster than that with long chain triacylglycerol (Siegel et al., 1985). There are composition differences between MOM and PDHM (Garcia-Lara et al., 2013), and while PDHM is associated with a lower incidence of feeding intolerance than formula (Boyd et al., 2007), the influence of PDHM on gastric emptying has not been studied.

The macronutrient composition of breastmilk after preterm birth differs from term breastmilk throughout the first 8 postnatal weeks (Bauer and Gerss, 2011). Since the ratio of whey to casein changes over the course of lactation (Lonnerdal, 2003), gastric curding of breastmilk also may vary with stages of lactation. Further, holder pasteurization of donor milk reduces fat and energy concentrations by approximately 3% (Garcia-Lara et al., 2013). PDHM is usually sourced from mothers of term infants >1 month postpartum therefore the composition is likely to differ from that of preterm MOM (Arslanoglu et al., 2013), which also may impact curding and gastric emptying in the preterm infant.

Commercial human milk fortifier (HMF) contains bovine milk proteins and other biochemical additives which have an acidifying effect that may lower fat absorption and reduces breastmilk protein by 14% (Erickson et al., 2013, Maggio et al., 2009). The composition of both breastmilk and HMF differ depending on the stage of lactation
and manufacturer respectively, with conflicting evidence regarding the effect of HMF on gastric emptying (Ewer and Yu, 1996, Gathwala et al., 2008, McClure and Newell, 1996, Yigit et al., 2008). This may be due to differences in milk composition that have not been quantified in previous studies.

Physicochemical differences in the characteristics of human and bovine milk proteins are likely to impact curd formation (West, 1986). Human casein forms a soft flocculent curd while bovine casein (even in whey dominant formula) forms a firm curd and this may explain differences in gastric emptying rates between breastmilk and bovine-based formula (Miller et al., 1990, West, 1986). While we have previously reported differences in curding between unfortified and fortified breastmilk, the influence of curd on gastric emptying has not been examined (Perrella et al., 2013).

Both pasteurization and the differing stages of lactation of donors compared to recipient infants’ mothers result in physicochemical differences between PDHM and MOM, yet no studies have investigated gastric emptying of PDHM. Similarly, despite wide variations between women, the effect of unfortified and fortified breastmilk composition on gastric emptying has not been examined in detail.

In this study we determined whether the biochemical compositions of fortified and unfortified MOM and PDHM feeds influenced gastric emptying and curding.

5.3 Methods

5.3.1 Participants

Medically stable infants born between 28-34 weeks gestation who were receiving full enteral feeds of MOM and/or PDHM were recruited from the Special Care Nurseries of King Edward Memorial Hospital, Western Australia. Infants with congenital abnormalities, gastrointestinal disease, or symptoms of feeding intolerance within the previous 24 hours were excluded from the study. For multiple birth infants, only one sibling was invited to participate in the study to prevent potential confounding of results.

The Ethics Committees of the Women and Newborn Health Service and The University of Western Australia gave approval for the study. Parents were provided
with verbal and written information about the study and provided signed informed consent for their infants’ participation in the study.

5.3.2 Study protocol

One pair of unfortified and fortified feeds was studied for each infant. Bovine-based FM 85 HMF (Nestlé Nutrition, Rhodes, NSW, Australia) was used to fortify feeds at the recommended dose of 5 g per 100 mL breastmilk and fortification status of the first of the paired feeds was determined by randomization. The inter-feed interval of was restricted to ≤72 hours. Paired feeds were matched for volume and feed frequency (2 or 3 hourly) with the infant lying in the same position (supine, right lateral, or prone) during feed delivery and throughout the postprandial period for both studied feeds. Each infant was scanned by ultrasound prior to, immediately after completion of feed delivery (T0), and at 30 minute intervals thereafter until the next feed was due ie. T90 for 2 hourly feeds and T150 for 3 hourly feeds, providing matched time points for comparison of stomach volume measurements. Infants were fed either MOM or PDHM, with PDHM studied only in those infants who were already receiving ≥2 PDHM feeds per day. The PDHM was supplied by the hospital’s human milk bank whereby breastmilk was sourced from women producing greater breastmilk volumes than that required by their own infant following either preterm or term birth, processed and pasteurized by the Holder method as per current recommendations (Human Milk Banking Association of North America, 2011, Hartmann et al., 2007).

Study feeds were scheduled to coincide with the infant’s usual feed times. A portable ultrasound machine was used to scan infants in their cot or incubator. Prior to feeding, nursing observations and infant cares were attended, the infant was placed in the supine position and infant clothing and bedding were adjusted to enable access to the upper abdomen for scanning. Sterile scan gel (PDI, New York) was pre-warmed in the infants’ incubator, or used at room temperature for infants in open cots. The intragastric tube was aspirated and tested for acidity to confirm correct tube placement. Immediately following the pre-feed scan, tube-fed infants were returned to the body position allocated by the clinician (prone, right lateral or supine) and the feed was delivered by gravity. The one bottle fed infant was fed prior to being returned to the cot in the supine position. The milk feeds were given at room
temperature. All infants maintained their body temperature within normal limits and tolerated the procedure well.

5.3.3 Ultrasound examination

The first author recorded serial ultrasound stomach images prior to and immediately following feed delivery and at 30 minute intervals until the next feed was due. A Sonologic SonoScape S6 (Brisbane, Australia) with the 5PI phased array transducer was used following the methods previously described (Perrella et al., 2013). Scan duration was less than 3 minutes at each time point. Images were recorded on the ultrasound machine and transferred to a laptop computer for measurement.

5.3.4 Ultrasound measurement of stomach volume

The direct ultrasound method was used whereby stomach volume calculations are performed using longitudinal, transverse and antero-posterior measurements of stomach images. This method has been assessed to have adequate repeatability and reliability (Perrella et al., 2013). Image measurements were made using ‘Screen Calipers’, V. 4.0 (Iconico Inc. New York, USA) and the stomach volume was calculated using the following equation:

\[
\text{Stomach volume: longitudinal} \times \text{antero-posterior} \times \text{transverse} \times 0.52
\]

(Lambrecht et al., 1988).

5.3.5 Echogenicity of stomach contents

At each time point the echogenicity of the stomach contents was assessed by rating the brightness of the longitudinal view of the stomach contents relative to that of the spleen as previously described, and were classified as having ‘higher’ or ‘lower’ echogenicity than the spleen (Perrella et al., 2013). Images with variable echogenicity were classified as ‘snow storm’ (predominately fluid filled; anechoic with small bright specks interspersed in the fluid), or ‘mixed’ (mixture of fluid and semi-solid contents; combination of lower and higher echogenic areas). Curd was classified with respect to its presence, density (high, medium or low compared to the spleen) and volume (high: occupied >50% of the stomach; low: <50%).
5.3.6 Analysis of feed composition

Unfortified milk samples were collected for both fortified and unfortified MOM and PDHM feeds. Samples of 3–5mL were collected from pooled expressed breastmilk on the morning of the study and placed in sterile polypropylene capped tubes (Sarstedt, Nümbrecht, Germany) stored in a refrigerator at 4°C then frozen at -20°C for later biochemical analysis.

Total protein, casein and whey were measured with the Biorad assay with casein and whey separated by the methods described by Kunz & Lonnerdal (Kunz and Lonnerdal, 1989, Kunz and Lonnerdal, 1990). Lactose was measured by the enzymatic spectrophotometric method (Arthur et al., 1989) and fat measured by the esterified fatty acid assay (Czank et al., 2009). Energy concentrations were determined using the calculation described by Neville & Jensen (Neville and Jensen, 1995). Biochemical analysis of MOM feeds containing HMF was not possible as, in the absence of manufacturer information regarding the specific mixture of amino acids and peptide bonds in HMF, it was not possible to validate laboratory assays for the hydrolyzed proteins in HMF. Therefore the total biochemical and energy concentrations of fortified feeds were calculated by adding the composition data provided by the HMF manufacturers to the measured unfortified MOM concentrations and expressed as grams per litre (g/L) and calories per 30 mL (cal/30mL) for each feed.

5.3.7 Statistics

5.3.7.1 Sample size determination

Initially recruitment of 2 groups of infants receiving either MOM or PDHM (n=40) was planned to obtain 20 pairs of unfortified and fortified feeds for each milk type. During recruitment, analysis of a previous study (Perrella et al., 2014) indicated that a sample size of 14 in each group was adequate to detect significant differences in gastric volume measurements between groups. As numbers already recruited to the study exceeded this, recruitment was stopped.

5.3.7.2 Statistical analysis

All analyses were performed using R 2.15.2 for Mac OSX (R Core Team, 2012). The nlme package was used for linear mixed modelling (Pinheiro et al., 2012).
Descriptive statistics are presented as mean±standard deviation (SD) range unless otherwise specified. P-values <0.05 were considered to be significant, with values less than 0.001 reported as <0.001. Predictors were retained in multivariate models only if significant at the p<0.05 level.

Both measured stomach volume and retained feed proportions were examined. Stomach volume measurements provide a precise measure of emptying during feed delivery while retained feed proportions allows clinically meaningful comparisons within and between infants.

The effects of milk type (MOM or PDHM) and fortification on stomach volumes and retained feed proportions at all postprandial time points were examined using linear mixed effects modelling with milk type and fortification status as predictors and individual infants as the grouping variable. For sequential measurements, time post-feed was also accounted for. To test for non-linear changes in post-feed time we considered time as a 2° polynomial based on our findings for a previous analysis of serial gastric volume measurements (Perrella et al., 2014). This was retained as the default in all models, testing the curve and trend separately. Covariates for the initial model were determined by identifying all predictors that were significant after accounting for time and milk type.

The final models were selected by sequentially omitting non-significant variables until all remaining variables had p-values <0.05, and then separately testing for significance of each of the omitted variables when added to this model. Where additional significant variables were found, this process was then repeated.

Considered covariates for influences on measured stomach volumes and retained feed proportions were: birth gestation, birth weight, size for gestational age, sex, postnatal age, corrected gestational age, the biochemical concentrations (g/L) of carbohydrate, lactose, protein, whey, casein, fat; feed energy value (cal/30mL), infant weight, feed volume, feed frequency, feed mL/kg, pacifier use during feed delivery, infant body position and continuous positive airway pressure (CPAP) use. Appropriateness of the models was assessed with visual inspection of residual plots.

Stomach volumes measured at T90 for 2 hourly feeds and at T150 for 3 hourly feeds were treated as final gastric residual volumes. Differences between milk types
and fortification status in frequencies of empty stomachs (volume=0mL), residual stomach volumes, and retained proportions >30% of delivered feed volume were tested for using Fisher’s Exact Test; these were performed separately for the two feed frequencies. Influences on residual stomach volumes and retained feed proportions were assessed separately for each feed frequency using linear mixed effects modelling with milk fortification status, feed volume and feed duration as predictors and individual infants as the grouping variable.

5.4 Results

5.4.1 Infant characteristics

A total of 32 infants (n=17 MOM; n=15 PDHM) were recruited. Complete data was attained for 62 of 64 feeds, with T150 measurements not available for 2 fortified feeds.

Infant (13 female, 19 male) characteristics were: birth gestation 29.6±1.4 weeks (28-32.9 weeks); birth weight 1271±233 g (895-1860g); corrected gestational age 32.5±1.2 weeks (30.4–36.1 weeks), weight 1515±284 g (1020–2200g) and postnatal age 20 days±8.4 (7–42 days). Five infants were small for gestational age (SGA). Age at commencement of full enteral feeds was 9 days±4 (2–20 days) and feed characteristics are reported in Table 5.1. Ten infants received nasal CPAP. Infant medications (including caffeine, vitamin D, ferrous sulfate, nystatin and probiotic Bifidobacterium breve M-16V) were administered to study infants at the prescribed times, none of which are known to influence gastric emptying. Infant characteristics were similar for those receiving MOM and PDHM other than those fed PDHM 2 hourly who were on average 13 days younger than their MOM fed counterparts (p=0.042, Table 5.1).
Table 5.1 Infant and feed characteristics for 2 hourly and 3 hourly unfortified and fortified feed pairs of mother’s own milk (MOM) and pasteurized donor human milk (PDHM) where one feed was fortified with FM-85 (Nestlé) human milk fortifier. Data are presented as mean±standard deviation, (range).

<table>
<thead>
<tr>
<th>Feed pairs</th>
<th>2 hourly feeds</th>
<th>3 hourly feeds</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOM</td>
<td>PDHM</td>
<td>MOM</td>
</tr>
<tr>
<td>n = 8</td>
<td>n = 11</td>
<td>n = 9</td>
<td>n = 4</td>
</tr>
<tr>
<td>Feed characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed volume (mL)</td>
<td>19±3 (14–22)</td>
<td>18±4 (9–23)</td>
<td>36±4 (30–42)</td>
</tr>
<tr>
<td>Prescribed feed (mL/kg)</td>
<td>14±1 (12–15)</td>
<td>13±2.0 (8–16)</td>
<td>20±1 (19–22)</td>
</tr>
<tr>
<td>Feed duration (min)</td>
<td>9±2 (5–12)</td>
<td>7±2.8 (4–11)</td>
<td>14±5 (8–21)</td>
</tr>
<tr>
<td>Infant characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected age (weeks)</td>
<td>32.3±0.6 (31.4–32.9)</td>
<td>31.7±1.4 (30.4–34.3)</td>
<td>34±1.5 (31.4–36.1)</td>
</tr>
<tr>
<td>Postnatal age (days)</td>
<td>19±7 (11–33)</td>
<td>18±6 (9–30)</td>
<td>29±10.0 (10–42)</td>
</tr>
<tr>
<td>Weight (grams)</td>
<td>1383±199 (1090–1728)</td>
<td>1327±183 (1020–1680)</td>
<td>1775±231 (1450–2200)</td>
</tr>
</tbody>
</table>

*p=0.042
5.4.2 Milk composition

There were significant differences in the biochemical compositions of MOM and PDHM (Table 5.2). MOM total protein and whey concentrations were higher, while casein, carbohydrate and lactose concentrations were lower than that of PDHM. The average casein concentration of MOM was one third of that of PDHM (MOM 2.4 g/L, PDHM 7.6 g/L, p<0.001). Fat and energy values were not different between milk types.

<table>
<thead>
<tr>
<th>Biochemical Concentration (g/L)</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM n=17</td>
<td>20±4.6</td>
<td>(12.6 – 29.1)</td>
</tr>
<tr>
<td>PDHM n=15</td>
<td>13.2±1.6*</td>
<td>(9.8 – 15.5)</td>
</tr>
<tr>
<td><strong>Whey</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM n=17</td>
<td>15.7±4.5</td>
<td>(9.2 – 24)</td>
</tr>
<tr>
<td>PDHM n=15</td>
<td>7.3±1.7*</td>
<td>(4.3 – 10.5)</td>
</tr>
<tr>
<td><strong>Casein</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM n=17</td>
<td>2.4±1.3</td>
<td>(0.6 – 5.7)</td>
</tr>
<tr>
<td>PDHM n=15</td>
<td>7.6±3.7**</td>
<td>(1.4 – 13.4)</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM n=17</td>
<td>53.9±4.1</td>
<td>(46.7 – 59)</td>
</tr>
<tr>
<td>PDHM n=15</td>
<td>65.9±7.9*</td>
<td>(56.5 – 58.5)</td>
</tr>
<tr>
<td><strong>Lactose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM n=17</td>
<td>53.9±4.1</td>
<td>(46.7 – 59)</td>
</tr>
<tr>
<td>PDHM n=15</td>
<td>65.9±7.9*</td>
<td>(56.5 – 58.5)</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM n=17</td>
<td>40±8.1</td>
<td>(17.5 – 60.6)</td>
</tr>
<tr>
<td>PDHM n=15</td>
<td>35.4±13.1</td>
<td>(14.3 – 59.7)</td>
</tr>
<tr>
<td><strong>Energy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM n=17</td>
<td>19.1±2.3</td>
<td>(16.2 – 24.7)</td>
</tr>
<tr>
<td>PDHM n=15</td>
<td>18.9±3.6</td>
<td>(12.6 – 25.1)</td>
</tr>
</tbody>
</table>

*p<0.001, **p=0.002

5.4.3 Gastric emptying during feeding

Lower immediate post-feed proportions of the delivered feed volume were associated with higher total casein, whey and lactose concentrations (Table 5.3). For each additional 1g/L of casein, the retained proportion was 4.4% lower, while for each additional 1g/L of whey and lactose the retained proportions were 2.8% and 1.4% lower respectively (all p<0.001). While half of the study feeds were fortified with a whey dominant HMF, further analysis using the unfortified milk composition data for
all feeds confirmed that the effect of whey was attributable solely to the human whey component.

When compared to unfortified MOM, retained proportions of fortified MOM and fortified PDHM feeds were 32% (p<0.01) and 23% (p=0.026) higher respectively. A summary of covariates associated with significant differences in immediate post-feed stomach volumes is presented in Table 5.3.

Advancing birth gestation was associated with a higher retained feed proportion, increasing by 1.2% (p<0.001) for each additional week of gestation, while for each additional 1 mL increase in feed mL/kg the retained proportion was reduced by 9.3% (p<0.001). SGA infants had retained proportions on average 16% lower than that of AGA infants (p=0.024). While proportions of retained feed did not differ with infant position, post feed stomach volumes were on average 5.5 mL larger for supine infants when compared to those positioned prone (p=0.024).

Males had retained feed proportions on average 17.6% lower (p<0.001) than female infants. Further analysis of infant gender, milk type and composition showed that the mean lactose concentration of PDHM fed to males (n=10, mean 67.9g/L) was higher than that fed to females (n=5, mean 61.8g/L, p=0.28) and significantly higher than that of MOM fed to males (n=9, 54.0g/L, p<0.001) and females (n=8, 53.7g/L, p<0.001).

5.4.4 Postprandial emptying

Influences on serial measures of postprandial gastric emptying included milk casein concentration, milk type and fortification. For each additional 1g/L of casein the retained feed proportions were 1.6% lower (p=0.009). Retained proportions of PDHM feeds were 15% higher (p<0.006) than that of MOM across the postprandial period. Slower gastric emptying was observed for feeds fortified with FM 85 HMF, with average retained proportions 7.0% higher for PDHM and 8.8% higher for MOM (both p<0.001).

The proportion of feed remaining in the stomach decreased over time (p=0.001) with the emptying rate progressively slowing (p<0.001). For each 1 mL increase in feed volume, there was a 1.3% increase in the proportion of feed retained (p<0.001, Table 3). Feed frequency and infant positioning influenced emptying. When
compared to 3 hourly feeds, 2 hourly feeds emptied more slowly with an average 33.3% higher feed proportion retained (p<0.001). Infants positioned supine retained an average 16% higher feed proportion than those positioned in the prone or right lateral positions (p<0.002, Table 5.3)

Figure 5.1 Gastric emptying curves for preterm infants fed paired feeds of unfortified and fortified mother’s own milk (MOM) or pasteurized donor human milk (PDHM). FM-85 (Nestlé) human milk fortifier (HMF) was used to fortify feeds.
Table 5.3 Significance (p-values) of selected predictors for measured stomach volumes (volume) and calculated proportions of delivered feed volume (% feed) immediately post feed and for subsequent serial postprandial time points after feeds of mothers own milk (MOM) and pasteurized donor human milk (PDHM). Variables were included in the table if they were composition variables, or if they were significant in at least one model. Bold text indicates p-values for the significant variables retained in the final model. All other p-values come from models containing the final model plus the specified predictor. Coefficients are presented only for variables in the final model. For categorical variables, these indicate the average difference between categories. For continuous variables, these indicate the average change per unit increase in the predictor.

<table>
<thead>
<tr>
<th>Feed type</th>
<th>Immediately post feed</th>
<th>Serial postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>volume (mL)</td>
<td>% feed</td>
</tr>
<tr>
<td></td>
<td>coefficient</td>
<td>p</td>
</tr>
<tr>
<td>Feed type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOM</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>PDHM</td>
<td>0.17</td>
<td>0.026</td>
</tr>
<tr>
<td>Feed fortification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfortified breastmilk</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>Breastmilk + FM 85</td>
<td>1.4</td>
<td>0.03</td>
</tr>
<tr>
<td>HMF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed composition g/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.36</td>
<td>not run*</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.36</td>
<td>-1.44</td>
</tr>
<tr>
<td>Fat</td>
<td>0.24</td>
<td>0.91</td>
</tr>
<tr>
<td>Protein</td>
<td>1.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Whey</td>
<td>0.62</td>
<td>-2.8</td>
</tr>
<tr>
<td>Casein</td>
<td>-0.6</td>
<td>0.048</td>
</tr>
<tr>
<td>Calories/30 mL</td>
<td>0.18</td>
<td>0.94</td>
</tr>
<tr>
<td>Covariates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Birth weight (g), appropriateness of growth for gestational age, postnatal age, corrected gestational age (weeks), infant weight, use of continuous positive airway pressure, pacifier use, and feed duration (min) did not significantly add to the model (p&gt;0.1) and have been omitted from this table.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.55</td>
<td>-17.6</td>
</tr>
<tr>
<td>Infant position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prone</td>
<td>5.5</td>
<td>0.024</td>
</tr>
<tr>
<td>Right lateral</td>
<td>0.72</td>
<td>0.34</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadratic term</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Feed volume</td>
<td>1.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Feed frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hourly</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>2 hourly</td>
<td>13.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Feed ml/kg</td>
<td>0.26</td>
<td>-9.3</td>
</tr>
<tr>
<td>Included interactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FM 85 HMF x PDHM</td>
<td>-1.89</td>
<td>0.003</td>
</tr>
<tr>
<td>2 hourly feed freq x time</td>
<td>52.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
5.4.5 Gastric residuals

Summaries of gastric residual data are presented in Table 5.4. Proportions of empty stomachs were similar between MOM and PDHM groups (2 hourly feeds p=0.18; 3 hourly feeds p=1.0) and between unfortified and fortified feeds (2 hourly feeds p=1.0; 3 hourly feeds p=0.21) but were observed less frequently in 2 hourly feeds (T90; 39%) than in 3 hourly feeds (T150; 62.5%).

Residual stomach volumes were similar between MOM and PDHM (2 hourly feeds 2.5±1.6 mL vs. 1.6±2.0 mL respectively, p=0.55; 3 hourly feeds 0.6±1.1 mL vs. 1.9 ±3.9 mL respectively, p=0.802), and were significantly higher for fortified feeds (2 hourly feeds average 1.1 mL higher, p=0.007; 3 hourly feeds average 1.5 mL higher, p=0.019). A trend to larger residual volumes following larger feed volumes was seen for 2 hourly feeds (p=0.052) only.

Residual stomach volumes >3 mL were more common for 2 hourly feeds (Table 4) and were associated with milk fortification (2 hourly feeds, p=0.007; 3 hourly feeds p=0.038) while milk type and feed duration were not significant.

Residual volumes >30% of delivered feed volume occurred only in 5 fortified feeds (4 of 2 hourly feeds; 1 of 3 hourly feeds, Table 5.4). For 2 hourly feeds, the effect of fortifier was significant (p=0.007) while feed volume and milk type were not associated with gastric residuals as retained feed proportions.
Table 5.4 Gastric residuals reported for all feed types. Counts of empty stomachs (stomach volume = 0 mL), residual volumes >0 mL, residual volumes >3 mL (Volume >3 mL) and volumes >30% of delivered feed volume (Volume >30% feed) for 2 hourly feeds at 90 min post feed and 3 hourly feeds at 150 min post feed.

<table>
<thead>
<tr>
<th></th>
<th>Unfortified</th>
<th></th>
<th>Fortified</th>
<th></th>
<th>All feeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOM</td>
<td>PDHM</td>
<td>MOM</td>
<td>PDHM</td>
<td>n=38</td>
</tr>
<tr>
<td>2 hourly feeds</td>
<td>n=8</td>
<td>n=11</td>
<td>n=8</td>
<td>n=11</td>
<td>n=38</td>
</tr>
<tr>
<td>Empty stomach</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Residual volume &gt;0 mL</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Volume &gt;3 mL</td>
<td>5</td>
<td>3</td>
<td>5*</td>
<td>4*</td>
<td>16</td>
</tr>
<tr>
<td>Volume &gt;30% feed</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3*</td>
<td>4</td>
</tr>
<tr>
<td>3 hourly feeds</td>
<td>n=9</td>
<td>n=4</td>
<td>n=7</td>
<td>n=4</td>
<td>n=24</td>
</tr>
<tr>
<td>Empty stomach</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Residual volume &gt;0 mL</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Volume &gt;3 mL</td>
<td>0</td>
<td>1</td>
<td>2*</td>
<td>1*</td>
<td>4</td>
</tr>
<tr>
<td>Volume &gt;30% feed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1*</td>
<td>1</td>
</tr>
</tbody>
</table>

PDHM = pasteurized donor human milk, MOM = mother’s own milk
Fortified feeds were fortified with FM-85 (Nestlé) human milk fortifier.
*p≤0.007 FM-85 fortifier associated with volume >3 mL in 2 hourly feeds, volume >30% feed volume in PDHM feeds
# p=0.038 FM-85 fortifier associated with volume >3 mL in 3 hourly feeds

5.4.6 Echogenicity of stomach ultrasound images

Frequencies of echogenicity ratings of stomach contents across the monitoring period are given in Figure 5.2. Echogenicity ratings were not related to milk casein concentration (p=0.11) or any of the other components measured (p≥0.28). Across all feeds, ratings of lower echogenicity were infrequent and mixed echogenicity was the most common rating. Snow storm ratings were most prevalent immediately after delivery of unfortified feeds and were not observed beyond T30 (Figure 5.2). Patterns of echogenicity were similar between MOM and PDHM although snow storm was less common in fortified PDHM.
**Figure 5.2** Serial ratings of gastric content echogenicity recorded immediately post feed (T0) and at 30 minute intervals thereafter for 2 hourly and 3 hourly feeds of unfortified and fortified mother’s own milk (MOM) and pasteurized donor human milk (PDHM). FM-85 (Nestlé) human milk fortifier (HMF) was used to fortify feeds.

**5.4.7 Presence, volume and density of gastric curd**

Gastric curd was identified in all but one longitudinal stomach image with a measurable stomach volume (309/310). Frequencies of gastric curd volume and density ratings across the monitoring period are given in Table 5.5.

Ratings of high curd volume increased over time for all feed types, and were more prevalent for fortified than unfortified feeds in the first 30 minutes following feed delivery. Curd volume ratings were similar between groups for 3 hourly feeds and were somewhat more variable for 2 hourly feeds (Table 5.5). Immediate post-feed ratings of high curd volume were associated with feeds of higher casein concentrations (p=0.014).
For all feed types, high curd density was the predominant curd density rating across all time points (Table 5.5). Immediate post-feed ratings of high curd density were associated with feeds of higher casein concentration \( (p=0.036) \).

**Table 5.5** Serial counts and proportions (%) of gastric curd volume and density ratings after 2 hourly and 3 hourly feeds of mother’s own milk (MOM) and pasteurized donor human milk (PDHM), both unfortified and fortified with FM-85 (Nestlé) human milk fortifier (HMF). Ratings were recorded immediately post feed (T0) and at 30 minute intervals thereafter.

<table>
<thead>
<tr>
<th>Curd volume %</th>
<th>Curd density</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>n</td>
</tr>
<tr>
<td>3 hourly feeds</td>
<td></td>
</tr>
<tr>
<td><strong>MOM</strong></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>8</td>
</tr>
<tr>
<td>T30</td>
<td>9</td>
</tr>
<tr>
<td>T60</td>
<td>8</td>
</tr>
<tr>
<td>T90</td>
<td>1</td>
</tr>
<tr>
<td>T120</td>
<td>4</td>
</tr>
<tr>
<td>T150</td>
<td>2</td>
</tr>
<tr>
<td><strong>PDHM</strong></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>4</td>
</tr>
<tr>
<td>T30</td>
<td>4</td>
</tr>
<tr>
<td>T60</td>
<td>4</td>
</tr>
<tr>
<td>T90</td>
<td>3</td>
</tr>
<tr>
<td>T120</td>
<td>3</td>
</tr>
<tr>
<td>T150</td>
<td>1</td>
</tr>
<tr>
<td><strong>MOM + HMF</strong></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>4</td>
</tr>
<tr>
<td>T30</td>
<td>9</td>
</tr>
<tr>
<td>T60</td>
<td>9</td>
</tr>
<tr>
<td>T90</td>
<td>6</td>
</tr>
<tr>
<td>T120</td>
<td>4</td>
</tr>
<tr>
<td>T150</td>
<td>4</td>
</tr>
<tr>
<td><strong>PDHM + HMF</strong></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>4</td>
</tr>
<tr>
<td>T30</td>
<td>4</td>
</tr>
<tr>
<td>T60</td>
<td>4</td>
</tr>
<tr>
<td>T90</td>
<td>3</td>
</tr>
<tr>
<td>T120</td>
<td>2</td>
</tr>
<tr>
<td>T150</td>
<td>2</td>
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</tbody>
</table>
## Table 5.5 cont’d

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>Curd volume %</th>
<th>Curd density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>2 hourly feeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MOM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>8</td>
<td>3 (37.5%)</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td>T30</td>
<td>8</td>
<td>2 (25%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>T60</td>
<td>8</td>
<td>2 (25%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>T90</td>
<td>6</td>
<td>-</td>
<td>6 (100%)</td>
</tr>
<tr>
<td><strong>PDHM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>11</td>
<td>7 (64%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td>T30</td>
<td>11</td>
<td>2 (18%)</td>
<td>9 (82%)</td>
</tr>
<tr>
<td>T60</td>
<td>11</td>
<td>-</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>T90</td>
<td>5</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td><strong>MOM + HMF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>8</td>
<td>5 (62.5%)</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>T30</td>
<td>8</td>
<td>1 (12.5%)</td>
<td>7 (87.5%)</td>
</tr>
<tr>
<td>T60</td>
<td>8</td>
<td>-</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>T90</td>
<td>6</td>
<td>-</td>
<td>6 (100%)</td>
</tr>
<tr>
<td><strong>PDHM + HMF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>11</td>
<td>5 (45.5%)</td>
<td>6 (54.5%)</td>
</tr>
<tr>
<td>T30</td>
<td>11</td>
<td>4 (36%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>T60</td>
<td>11</td>
<td>2 (18%)</td>
<td>9 (82%)</td>
</tr>
<tr>
<td>T90</td>
<td>6</td>
<td>1 (17%)</td>
<td>5 (83%)</td>
</tr>
</tbody>
</table>

* No visible curd for one T0 image

### 5.5 Discussion

This study has found that PDHM empties more slowly than MOM and that feeds with higher casein, lactose and whey concentrations are associated with faster gastric emptying. Further, breastmilk fortified with FM 85 empties more slowly than unfortified breastmilk. While statistically significant, the magnitudes of the effects of PDHM and feed composition differences on retained feed proportions are small and unlikely to be of clinical concern for the stable preterm infant.

In this first comparison of gastric emptying between PDHM and MOM it was found that gastric emptying of PDHM was slower than that of MOM both during feed
delivery and postprandially. PDHM the immediate post feed retained feed proportion was on average 23% higher than MOM, while for all subsequent postprandial time points this reduced to 15%. The rate of emptying of PDHM is closer to that of MOM than infant formula, with calculations based on published data indicating that postprandial retained proportions of formula are in the range of 23% to 29% higher than that of breastmilk in preterm infants (Cavell, 1982, Ewer et al., 1994). Final stomach residual volumes were not different between milk types indicating that the same end point was met for both PDHM and MOM (Table 5.4, Figure 5.1). Further final gastric residuals were typically 12% to 22% of the feed volume, which is below the 30% to 50% that is typically considered a marker of feeding intolerance (Moore and Wilson, 2011) and supports clinical observations and evidence that PDHM is unlikely to be implicated in large residuals associated with feeding intolerance (Boyd et al., 2007).

Compositional differences between PDHM and MOM did not explain the disparity in gastric emptying seen in this study. Higher human casein concentrations are associated with faster emptying (Perrella et al., In press) while an association between lactose concentration and gastric emptying has not previously been reported. Yet feeds of PDHM emptied more slowly than MOM despite having higher concentrations of casein and lactose. It is possible that the effect of pasteurization or frozen storage alters components of donor human milk and so counters the effects of casein and lactose on emptying. Holder pasteurization causes complete inactivation of the BSSL and lipoprotein lipase (Henderson et al., 1998). BSSL enables almost complete hydrolysis of breastmilk triacylglycerol through its synergistic action with pancreatic lipase-related protein 2 (Lindquist and Hernell, 2010). As the presence of lipids in the duodenum and ileum slows gastric emptying through triggering of the ileal brake, it is possible that restricted lipolysis of pasteurized milk is a mechanism for slower emptying of PDHM (Feltrin et al., 2004). Also, heat treatment of bovine milk has been shown to alter the structure of whey proteins resulting in interactions between denatured whey proteins and casein micelles (Vasbinder and de Kruif, 2003). It is therefore conceivable that Holder pasteurization creates similar interactions in breastmilk possibly negating the faster gastric emptying associated with higher human casein concentrations.
Higher concentrations of casein, whey and lactose were significantly associated with faster emptying of breastmilk although the effect sizes were small. In this study unfortified PDHM had significantly higher mean casein and lactose concentrations and lower mean total protein and whey concentrations than unfortified MOM (Table 5.3), with breastmilk composition likely reflecting the later lactation stages of milk donors (Bauer and Gerss, 2011). Higher concentrations of human casein were associated with faster gastric emptying both during and after feed delivery (Table 5.3). Previously we detected an association between higher casein concentrations and more rapid emptying during feed delivery (Perrella et al., In press). The lack of effect on postprandial emptying may be explained by the inclusion of feeds fortified with a HMF containing partially hydrolysed bovine casein whereby bovine casein may have impeded subsequent gastric emptying (Miller et al., 1990). Reports of the effects of human casein on gastric emptying are limited to a study of term breastfed infants whereby no effect of casein was observed (Khan et al., 2013, Khan, 2012a). However the range of observed casein concentrations was narrow ie. 2–5g/L compared to 0.6–13.4g/L in this study, and so would preclude examination of the effects of high casein concentrations.

Gastric emptying and enterogastrone responses differ according to the physicochemical structure of proteins such that ingested bovine peptide hydrolysate is associated with greater GIP secretion in adults. Human caseins in preterm milk are hydrolysed to a greater extent than whey proteins and bovine casein (Miller et al., 1990, Armaforte et al., 2010). It is possible that human casein facilitates gastric emptying rather than triggering the ileal brake and may explain in part the more rapid emptying of breastmilk feeds when compared to formula feeds (Cavell, 1981, Van Den Driessche et al., 1999). While human casein concentrations vary widely between women (Mitoulas et al., 2002) the small magnitude of the effect of higher casein concentrations on gastric emptying is unlikely to be clinically important when breastmilk feeds have casein concentrations within the expected range.

Similarly small effect sizes were seen with higher lactose and whey concentrations and faster gastric emptying during feed delivery (Table 5.3). Preterm MOM concentrations of lactose were lower and whey higher than PDHM. It is possible that whey is a more potent facilitator of gastric emptying than lactose, and so the
higher whey concentrations explain in part the faster emptying during feed delivery when compared to PDHM (Bauer and Gerss, 2011).

The faster gastric emptying during feed delivery seen with male infants is explained by the lactose concentrations of PDHM fed to male infants that were on average 6.1g/L and 14.2g/L higher than that of PDHM and MOM fed to females. Reductions in retained proportions of 1.4% per additional 1g/L of lactose would result in post feed proportions on average 8.5% and 19.9% lower for males, contributing to the lower post feed retained proportions observed in males. Therefore a gender difference in gastric emptying is unlikely.

The energy concentration of breastmilk feeds did not influence gastric emptying with energy concentrations ranging from 12.6–30.4 kcal/30 mL. There is limited evidence of a relationship between energy concentration and gastric emptying in preterm infants. Similar rates of emptying have been observed for breastmilk or formula feeds of 5-20 kcal/30 mL (Ramirez et al., 2006), while increasing energy concentrations have been associated with slower postprandial emptying for formula feeds of 5-20kcal/30 mL from 20 minutes post feed, with differences between 20kcal and 24kcal noted only at 80 minutes post feed (Siegel et al., 1984). Results from this and previous studies suggest that the infant’s gastric emptying response to increasing energy concentrations is diminished, and this may serve to facilitate the physiological emptying of fat-rich breastmilk that is ingested towards the end of a breastfeed (Khan et al., 2013). Also, the absence of an effect of energy concentrations observed in our sample suggest that other biochemical factors are responsible for the slower rate of emptying associated with some human milk fortifiers.

Fortification with FM 85 HMF was associated with slower gastric emptying across all time points, with the magnitude of the effect smaller for PDHM than for MOM. This finding concurs with Ewer & Yu who reported an average gastric half emptying time of FM 85 fortified MOM to be 48 minutes, more than double the 21 minute average for unfortified MOM (Ewer and Yu, 1996). Interestingly we found no association between the biochemical components of FM 85 HMF and the increased retained feed proportion. It is likely that other factors associated with fortification, such as increased osmotic concentration above the recommended maximum of 400 mOsmol/L, which has been documented with FM 85 HMF (>472 mOsm/L) may have
contributed to slower gastric emptying (Kreissl et al., 2013). However, in terms of physiological significance the magnitude of the difference in retained feed proportions is not considered to be of clinical concern for stable preterm infants receiving MOM or PDHM feeds.

Supine infant positioning was associated with slower gastric emptying across the postprandial period with the retained feed proportion on average 16% higher than that of infants positioned prone or right lateral. Also for 3 hourly fed infants, a low proportion (22%) of those with an empty stomach at 2 hours post feed were positioned supine compared to 69% of those with a measurable stomach volume. The observed inter-individual effects of positioning on gastric emptying are consistent with several published studies (Chen et al., 2013, Cohen et al., 2004, Yu, 1975) and further support the use of positioning as a conservative clinical management strategy for feeding intolerance.

Echogenicity and curding ratings were similar for MOM and PDHM, and reflected gastric emptying with postprandial echogenicity ratings generally indicating higher fluid proportions (snow storm and mixed), subsequently progressing to predominately curd (mixed and high echogenicity) as the fluid portion emptied from the stomach. These findings are congruent with previous observations (Perrella et al., 2014).

Immediate post feed ratings of high curd density and high curd volume were associated with higher casein concentration, and HMF also significantly contributed to high curd volume (Table 5.5). These finding also concur with our previous findings (Perrella et al., 2013) with HMF providing a more acidic environment that facilitates precipitation (Erickson et al., 2013). Published data regarding curding is largely limited to animal studies. While influenced by feed composition, we found no associations between post feed echogenicity and curding ratings and gastric emptying, and so increased curding does not explain the slower emptying of fortified milk feeds.

A gastric residual volume $\leq 2.5mL$ that is predominantly curd appears to be usual for stable preterm infants (Perrella et al., In press), with most (62%) 3 hourly feeds resulting in empty stomachs. Gastric residual feed proportions were similar between MOM and PDHM, and were 10% and 15% for unfortified and fortified feeds.
respectively. These are similar to the 15% and 16% residual feed proportions reported for breastmilk or infant formula (Malhotra et al., 1992, Cobb et al., 2004) and below the 30%-50% considered to be a sign of feeding intolerance (Moore and Wilson, 2011).

5.6 Conclusion

Higher concentrations of casein, lactose and whey facilitate gastric emptying, while Holder pasteurization and fortification each slow gastric emptying minimally. As the magnitude of difference on gastric emptying are small, it is likely the differences between MOM and PDHM are not great enough to contribute to feeding intolerance. Similarly the use of HMF does not cause clinically significant increases in gastric residual volumes for the stable preterm infant.
Chapter 6 General Discussion

Monitoring of gastric emptying plays an important role in identifying feeding intolerance and impending gastrointestinal disease in preterm infants. Delayed gastric emptying is associated with clinical signs such as vomiting, a distended abdomen and/or large gastric residual volumes (Moore and Wilson, 2011) and may herald a range of conditions from benign feeding intolerance, to ileus secondary to sepsis or fulminating NEC (Bertino et al., 2009, Cobb et al., 2004, Gonzalez et al., 2003, Lin and Stoll, 2006, Moore and Wilson, 2011). Current measures of gastric emptying are restricted to basic observations of the clinical signs of delayed emptying (Fanaro, 2013, Lucchini et al., 2011), as well as gastric aspirate measurements that are not reliable (Metheny et al., 2005, Moore and Wilson, 2011).

Normal patterns of gastric emptying in preterm infants have not been established. Without highly accurate measures it is not possible to attain these, nor evaluate the effects of feed composition and volume, infant acuity and maturity on emptying. There is a pressing need for a sensitive non-invasive measure of gastric emptying that may contribute to the establishment of normal parameters, evaluation of influences on emptying and advancement of knowledge regarding pathologies associated with delayed gastric emptying.

While great progress has been made in the monitoring and management of neonatal cardiovascular and respiratory disease over the last 30 years (Allen et al., 2011, Fanaroff et al., 2007), advances with regard to gastrointestinal complications have been limited. Feeding intolerance and NEC remain significant contributors to preterm infant morbidity (Fanaroff et al., 2007), yet further understanding of the aetiologies of both conditions is urgently needed. Some progress has been made in identifying antecedents and protective factors, with recent evidence suggesting that aberrant gut microbiota and/or low breastmilk levels of the cytokine transforming growth factor β may contribute (Di Mauro et al., 2013, Frost et al., 2014), while breastmilk feeding as well as specific probiotic strains reduce the incidence of NEC (Mihatsch et al., 2012, Wang et al., 2012, AlFaleh and Anabrees, 2014) and feeding intolerance (Indrio et al., 2008, Rojas et al., 2012, Oncel et al., 2014). Compared to the wide array of monitoring methods and investigations available for the detection of
cardiorespiratory complications, detection of gastrointestinal complications is rudimentary. That is, clinical suspicion of feeding intolerance and NEC is largely based on the recognition of gross clinical signs such as large gastric aspirates, vomiting and abdominal distension (Moore and Wilson, 2011), while diagnostic radiology typically provides confirmation of NEC when the infant is already moderately to critically ill (Epelman et al., 2007). It is imperative that progress be made in the development of sensitive and clinically accessible tools to identify precursors to clinical signs of disease, and to monitor gastrointestinal complications in the preterm population.

In this project I have utilized ultrasound to clearly demonstrate that calculation of stomach volumes from gastric ultrasound images provides a highly precise, reliable, safe, non-invasive and accessible method of monitoring gastric emptying in preterm infants that is accurate to within 1 mL (chapter 1). Unlike all other reported methods, the ultrasound method simultaneously measures gastric emptying during feed delivery, accounts for both solid and liquid portions of stomach contents, enables evaluation of gastric curding and importantly provides a direct volume measurement that is essential to the evaluation of large gastric residuals, which are associated with feeding intolerance and NEC. The gastric emptying curves generated from serial stomach volume measurements (Chapters 3-5: Figures 4, 5, 6) are similar to those determined from other methods enabling comparison of results (Chen et al., 2013, Ewer et al., 1994, Ewer and Yu, 1996, Yigit et al., 2008). Notably, however the ultrasound method quantifies the degree of emptying that occurs during feed delivery, a factor that is typically not accounted for by other methods. Therefore most previously published results will have overestimated the gastric half emptying time by an average of 16% (range 5 to ≥30%), and these inaccurate estimations can result in misdiagnosis of delayed gastric emptying (Lin et al., 2000).

When implementing monitoring of gastric emptying, clinically, it is critical that there is confidence not only in the accuracy of the measurement, but also in the clinical relevance of the measurements. To ensure this I have for the first time monitored gastric emptying sequentially in the same infant with the same feed composition and volume to determine whether the gastric emptying measurements for one feed are indicative of subsequent feeds. Previously repeated feeds were monitored at different days or weeks of age (Barbosa et al., 2005, Barnett et al., 1999,
Pozler et al., 2003). Fortunately I found that gastric emptying patterns are highly repeatable at least in stable preterm infants (chapter 2). Therefore, application of the ultrasound method offers the opportunity to establish reference gastric emptying curves for preterm infants similar to that available for the paediatric population (Heyman, 1998), providing an evidence based standard against which gastric emptying of infants with signs of feeding intolerance can be evaluated. Given that emptying patterns are consistent within an infant (Chapter 3, Figure 3.1) any deviation from the ‘norm’ might be an indicator of impending gastrointestinal complications.

The ability to examine intragastric contents may also provide insight into the digestion of different types of feeds as well as pathologies such as lactobezoar. Lactobezoar is a sporadic complication whereby formation of a firm intragastric coagulum of milk and mucous causes feeding intolerance, gastric outlet or intestinal obstruction or rarely, perforation (Heinz-Erian et al., 2012, Stanger et al., 2014, Schreiner et al., 1982, Stotzer et al., 1999). It is most common in preterm infants, and is associated with the use of casein dominant formula and HMF. It can be identified readily on ultrasound as a hyperechoic mass with well defined borders and mixed echogenicity (Naik et al., 1987). While infrequently reported, lactobezoar is potentially an under-diagnosed pathology that contributes to feeding intolerance in the preterm population (Stanger et al., 2014). A simple ultrasound scan may at least eliminate the presence of this pathology (Naik et al., 1987).

Despite wide differences in the composition of breastmilk both within and between women, it is surprising that the influence of breastmilk biochemical composition on gastric emptying has not been investigated. This is vitally important when assessing fortified feeds as standard fortification may confound the results. For standard fortification a set dose of HMF is added based on assumed breastmilk composition values, results in wide ranging (and often nutritionally suboptimal) feed composition values (Tables 4.2, 5.2 (Arslanoglu et al., 2010, de Halleux and Rigo, 2013, Rochow et al., 2013, Corvaglia et al., 2010)). In carrying out comprehensive studies accounting for milk composition, fortification and infant position I was able to show that PDHM (which had a different composition to MOM) and the use of FM-85 HMF slowed gastric emptying significantly. Whilst the differences were statistically significant (p range 0.026 to <0.001) they are unlikely to be clinically significant.
Nevertheless the sensitivity of the technique should not be understated. Bedside sonographic monitoring of gastric emptying would enable clinicians, for example, to evaluate the effect of new nutritional products including preterm formulae and HMF products on gastric curding and feeding tolerance under local clinical conditions.

Our more detailed biochemical analysis showed that higher concentrations of human casein, whey and lactose in breastmilk are associated with faster gastric emptying although the sizes of the effects were small (chapters 4, 5). Human milk proteins facilitate emptying as opposed to bovine proteins that are thought to inhibit gastric emptying due to formation of a more dense curd (Miller et al., 1990). We observed a wide range of protein concentrations in unfortified breastmilk, which despite fortification resulted in variable protein intake. Compared to the recommended protein intake of 5.25–6.0 g/day for a 1500g infant (Agostoni et al., 2010), standard fortification resulted in intakes of 5.5-8.9 g/day for fortified MOM and 4.6 – 5.7g for fortified PDHM (Table 5.2). Indeed wide ranges were observed for energy and all macronutrient concentrations in both MOM and PDHM groups, demonstrating disparities between assumed and actual nutrient intake for many infants. This is of clinical importance as under- and over-nutrition has consequences, with inadequate protein and energy intake major contributors to postnatal growth restriction and subsequent neurodevelopmental outcomes in very preterm infants (Embleton et al., 2001, Hay and Thureen, 2010, Stephens et al., 2009, Su, 2014). Our findings support the replacement of standard fortification with an individualised approach to improve postnatal weight gain, either through targeted fortification in response to milk analysis, or adjustable fortification in response to the infant’s metabolic response to protein intake (Alan et al., 2013, Arslanoglu et al., 2010, de Halleux and Rigo, 2013, Rochow et al., 2013).

In the absence of MOM, PDHM feeds provide infants with the preferred source of nutrition and a reduced risk of late onset sepsis (Cossey et al., 2013), feeding intolerance and NEC (Boyd et al., 2007). However, fat and energy content, and many bioactive and immunoprotective factors in breastmilk are altered by pasteurisation (Garcia-Lara et al., 2013) resulting in slower weight gain when compared to those fed unpasteurised milk (Andersson et al., 2007). We have shown that gastric emptying of PDHM is somewhat slower than MOM (chapter 5), however the degree of delay is not
clinically relevant for the stable preterm infant. Therefore evidence from this study supports current recommendations to feed fortified PDHM rather than preterm formula in the absence of MOM.

Differences in emptying of PDHM and MOM were not explained by macronutrient composition (Table 5.3). In fact, mean PDHM casein and lactose concentrations were significantly higher while whey concentrations were significantly lower than that of MOM, with each of these nutrients associated with faster emptying (Table 5.2). It is possible that pasteurisation alters interactions between casein and whey (Vasbinder and de Kruijff, 2003), which affects subsequent stimulation of the ileal brake. Pasteurisation also impacts cellular and immunoprotective components of breastmilk resulting in the inactivation of B cells, T cells and breastmilk lipases, reduced activity of lysozyme (74%) and lactoperoxidase (88%) and significantly reduced concentrations of lysozyme (60-70%), lactoferrin (44% – 66%), lactoperoxidase (82%) and secretory immunoglobulin A (sIgA, 26 – 79%) (Chang et al., 2013, Akinbi et al., 2010). Clearly optimisation of pasteurisation to both inactivate potentially harmful microorganisms and retain or minimise the loss of beneficial components would be ideal in order to preserve the benefits of breastmilk and possibly influence gastric emptying.

Ultraviolet radiation (UV-C, 250 – 270nm) has recently been researched as a potential method to inactivate potential pathogens in breastmilk while preserving various immunoprotective and bioactive components (Christen et al., 2013a, Christen et al., 2013b). Reduction of bacterial counts (E coli, S. aureus, S. epidermidis, E. cloacae) to acceptable levels was achieved whilst retaining significant proportions of lactoferrin, lysosome and sIgA (87%, 75% and 89%, respectively) compared to holder pasteurised milk (9%, 41% and 49%, respectively)(Christen et al., 2013a). Importantly, BSSL activity is conserved with UV-C treatment but completely inactivated by holder pasteurisation (Christen et al., 2013b, Henderson et al., 1998) and so its preservation is likely to overcome the problems of reduced fat absorption and slower weight gain associated with PDHM (Andersson et al., 2007). As UV-C does not involve heat, it is likely that macronutrient composition and whey-casein interactions of breastmilk are unaffected, and so gastric emptying would be expected to be similar to that of untreated milk. The inclusion of sonographic monitoring of gastric emptying in clinical
trials of UV-C treated breastmilk will contribute to knowledge of the effects of this treatment on gastric emptying and feeding tolerance (Simmer and Hartmann, 2009).

Gastric residual volumes have been implicated in feeding intolerance and NEC with residuals more than 30% to 50% of the previous feed being considered problematic (Lucchini et al., 2011, Moore and Wilson, 2011). Ultrasound imaging can facilitate rapid assessment of pre-feed residual volumes in infants exhibiting symptoms of feeding intolerance. This procedure is analogous to the hand held bladder scanning device that measures urinary retention volumes to avoid unnecessary bladder catheterisation and the associated risk of infection (Palese et al., 2010). In terms of the stomach a systematic review was recently completed on the sonographic assessment of gastric volume and content in pre-operative adults as a tool for determining aspiration risk associated with anaesthesia (Van de Putte and Perlas, 2014). Sonographic methods of calculating gastric volumes correlated with known ingested volumes, MRI volume calculations, gastroscope suction volumes and volumes aspirated via nasogastric tube, with qualitative descriptions of gastric contents contributing to the clinical assessment. It was concluded that gastric sonography offers a useful bedside screening tool that will inform anaesthetic management. Similarly, sonographic measurement of gastric residuals in preterm and post-operative infants might also be valuable in guiding the progression to full enteral feeds as well as accurately diagnosing large residual volumes.

Gastric residuals are routinely measured by aspiration of the infant’s intragastric tube. However correct placement of the tube, and therefore aspirated fluid volumes, are not consistently accurate (de Boer et al., 2009, Freeman et al., 2012) and may impact on the assessment of feeding tolerance. Ultrasound presents an alternative option for confirmation of correct positioning of the intragastric tubes in preterm infants. Two studies have compared bedside sonography and radiography as methods of confirming tube placement in adults, with a reported positive predictive value 97.4% (Kim et al., 2012) and sensitivity 97% (Vigneau et al., 2005) for sonography. These studies have used weighted intragastric tubes whereby a radiopaque material is contained within the tip of the tube to aid radiographic identification. Sonographic detection of non-weighted tubes is described in two case
reports including that of a correctly placed tube in an adult (Nguyen et al., 2012), and of a malpositioned tube in a preterm infant (Maruyama et al., 2003), while a letter to the editor describes an audit in which the non-weighted tube was identified in only one of ten preterm infants (Tamhne et al., 2006). Further exploration of the use of sonography for identifying correct intragastric tube placement in preterm infants is warranted.

It is interesting that a large proportion of infants in our studies had an empty stomach prior to the next feed that may coincide with cuing ‘early’ for next feed (average 2 hourly feeds 29%, 3 hourly feeds 63%). This in itself suggests many preterm infants would tolerate more frequent feeding and potentially increased nutrient intake. Further, semi-demand feeding may offer a suitable strategy for infants transitioning to oral feeds, whereby infants are fed according to a schedule, but are offered an oral feed earlier if cuing for a feed or are tube fed if sleeping when the scheduled feed is due (McCormick et al., 2010). There is some evidence that semi-demand fed preterm infants attain full oral feeds earlier (McCain et al., 2012) and are discharged at a lower postmenstrual age (average 4 days) than infants fed to a schedule (McCormick et al., 2010).

Confirmation of a diagnosis of NEC is traditionally based on the presence of specific patterns of intestinal gas visualised on abdominal radiographs such as intramural, portal vein and/or free intra-peritoneal gas, and these signs are only apparent when there is definite or advanced disease (Bell et al., 1978). Sonography enables identification of the same diagnostic signs as well as additional clinically relevant information such as echogenicity, perfusion and thickness of the bowel wall, peristalsis, presence of dilated bowel loops, and presence, volume and echogenicity of intra-abdominal fluid (Epelman et al., 2007). Increased bowel wall echogenicity and thickness, the presence of focal intra-abdominal fluid collections and the presence of echogenic free fluid are significantly predictive of adverse outcomes from NEC, while anechoic free fluid is not associated with NEC and therefore predictive of a positive outcome (Muchantef et al., 2013, Silva et al., 2013). Sonography is considered superior to radiography with regard to the early identification of intestinal perforation through the detection of echogenic free fluid (Dilli et al., 2011). Rapid assessment of bowel
viability and detection of impending bowel perforation is vital in expediting surgery for these critically ill infants.

Early recognition of NEC and differentiation from other acquired neonatal intestinal diseases or benign feeding intolerance however remains problematic (Gordon et al., 2012). There is conflicting evidence as to the usefulness of Doppler studies of superior mesenteric artery (SMA) blood flow in predicting feeding intolerance and NEC (Bora et al., 2009, Louis et al., 2013, Murdoch et al., 2006). However these studies have typically been performed within 24 hours of birth and the onset of NEC is on average 7 days for ‘early onset’ and 32 days for ‘late onset’ NEC (Yee et al., 2012). It is feasible that antecedents to NEC develop in the hours or days prior to its onset. For example, a reduction in portal vein blood flow in preterm infants has been associated with subsequent gastrointestinal symptoms in the first postnatal month (Kobayashi et al., 2014). The search for associations between NEC and measures of gastrointestinal physiology (such as gastric emptying and SMA blood flow in response to feeding) may be more fruitful if conducted around the time of onset of disease.

Additional to the calculation of residual volumes and monitoring of gastric emptying ultrasound offers a valuable adjunct to radiology that has the potential to alert the clinician to impending NEC, and assist in differential diagnosis, and in making timely judgments regarding surgical intervention and prognoses for infants with NEC.

Of the various methods available to measure gastric volume, calculation of stomach volumes from ultrasound images is the only method that possesses all of the desirable attributes of a neonatal screening and monitoring tool: it can be performed at the bedside, is repeatable, reliable and safe, provides real time results can be performed and interpreted by clinical staff, and is suitable for use in the critically ill preterm infant. Ultrasound is also a well accepted, safe and accessible modality in the neonatal setting whereby scanning is performed by the bedside to facilitate rapid diagnosis and management (Evans et al., 2011). Indeed over a decade ago ultrasound imaging was described as “...an essential part of the evaluation and delivery of care for most infants” (Burdjalov et al., 2002). Routine applications of ultrasound in neonatal care include serial cranial ultrasound to screen for and monitor intracranial haemorrhage and predict subsequent neurological outcomes (Amess et al., 2009), and
functional echocardiography to identify, monitor and inform the medical management of a range of cardiovascular complications including patent ductus arteriosus and persistent pulmonary hypertension of the newborn (Okazaki et al., 2003). Sonography offers an exciting opportunity to detect and monitor complications of the gastrointestinal tract with greater precision and at earlier stages than is currently possible.

6.1.1 Summary

My comprehensive studies have made a significant contribution to both the monitoring and understanding of gastric emptying in the preterm infant. We have validated a highly accurate, safe and accessible sonographic method for bedside monitoring of gastric emptying, curding and gastric residuals in preterm infants. For the first time we have established repeatability of gastric emptying in stable preterm infants providing confidence that a single measurement is representative for individual infants, and any subsequent deviations likely herald significant changes in the biochemical composition of feeds, or in gastrointestinal health. This validated bedside assessment method can potentially aid the clinician in monitoring gastric residuals in post surgical and feed intolerant infants. Further, sonography may inform nutritional management through in vivo evaluation of the tolerance of new pasteurisation treatments, HMF and formula products, and of feeding strategies such as semi-demand compared to scheduled feeds.

Through analysis of feed composition and serial postprandial tracking of gastric volumes, we have for the first time established the effects of feed biochemical composition on gastric emptying in preterm infants. The statistically significant but very small effects of lactose, casein and whey concentrations on emptying confirm that despite a diverse range of macronutrient compositions observed in MOM and PDHM, breastmilk is well tolerated and provides the optimal source of nutrition for infants. Similarly, pasteurisation slows gastric emptying but not to a degree that impacts feeding tolerance of breastmilk in the stable infant. The addition of FM-85 HMF slows emptying, most likely due to an osmotic load higher than that reported by the manufacturer. There is great potential value in clinical evaluation of nutritional products as in vivo outcomes may differ from theoretical assumptions.
Sonographic monitoring of the preterm gastric emptying and residual volumes, in conjunction with other gastrointestinal tract sonographic tools (such as imaging and Doppler studies) presents an exciting opportunity to develop better understanding of the aetiology of feeding intolerance, and more sensitive measures of NEC. Both feeding intolerance and NEC are universal challenges in neonatal care, and ultrasound is widely available. My studies demonstrate that the application of sonographic monitoring in the preterm infant has the potential to rapidly detect, monitor and respond to pathologies and therefore assist with the development of evidence-based treatments.
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