Impact of a baby-led approach to complementary feeding on iron and zinc intake and status: 
A randomised controlled trial

Lisa Daniels

A thesis submitted for the degree of

Doctor of Philosophy

University of Otago, Dunedin, New Zealand

2017
Abstract

Background: Baby-Led Weaning (BLW) is an approach to complementary feeding that is gaining popularity amongst parents worldwide. In this alternative approach to traditional spoon-feeding, infants feed themselves all of their food from the start of complementary feeding, which means that foods offered need to be finger foods that they can hold themselves. Although there are several proposed advantages of BLW, health professionals have expressed some concerns. Iron and zinc are a particular concern because commonly introduced ‘first foods’ that are easily picked up, such as fruits and vegetables, tend to be low in iron and zinc. No studies have yet investigated the impact of a baby-led approach to complementary feeding on biochemical iron and zinc status. Further to this, no studies have determined potentially modifiable ‘predictors’ of zinc status in toddlers from high-income countries.

Objectives: The overall aim of the Baby-led Introduction to SolidS (BLISS) randomised controlled trial was to determine whether a modified version of BLW prevents young children from becoming overweight, without increasing their risk of iron deficiency, growth faltering, and choking. The aim of this thesis was to determine the impact of this version of BLW modified to prevent iron deficiency, on iron and zinc intakes and status, and to determine potentially modifiable ‘predictors’ of zinc status in toddlers.

Methods: A total of 206 participants were randomised to Control or BLISS groups. Both groups received standard Well Child care from before birth. The BLISS group received eight additional visits (from before birth to 9 months) providing education and support on following the BLISS approach (i.e. BLW modified to increase iron intake). Weighed three-day diet records were used to assess the intake of key nutrients at 7 and 12 months, and a blood sample was collected using trace-element free techniques to determine biochemical iron
and zinc status at 12 months. Multiple regression analysis was used for the outcomes of the randomised controlled trial following the principles of modified intention to treat. ‘Predictors’ of zinc status were determined by univariate, then stepwise linear bootstrap, and multivariable regression analysis.

**Results:** There was no evidence of a difference in dietary iron and zinc intakes between the groups at 7 or 12 months (all \(p>0.42\)). However, there was a high prevalence of inadequate iron intakes (7 months: 74%, 12 months: 23-36%). There were no statistically significant differences in plasma ferritin (median: 29 μg/L Control, 27 μg/L BLISS; difference -2.6 μg/L; 95% CI -10.9, 5.8; \(p=0.55\)) or plasma zinc (mean: 9.6 μmol/L Control and BLISS; difference -0.09 μmol/L; 95% CI -0.67, 0.48; \(p=0.75\)) concentrations between the groups at 12 months. The majority (83%) of toddlers were iron sufficient, although a high proportion had low plasma zinc concentrations (63% Control, 57% BLISS). Red meat intake \((p=0.028)\), infant formula intake \((p=0.009)\), and food fussiness \((p=0.021)\) were statistically significant ‘predictors’ of plasma zinc concentration at 12 months.

**Conclusions:** These results suggest that a baby-led approach to complementary feeding does not appear to increase the risk of iron or zinc deficiency when parents are given advice to offer ‘high-iron’ foods at every meal. It is important to note, however, that this study assessed a modified version of BLW so no conclusions can be made about the risk of iron and zinc deficiency in infants following unmodified BLW. Red meat intake, infant formula intake, and food fussiness were all significant ‘predictors’ of zinc status at 12 months. Of particular interest is the association with food fussiness, and further research should investigate whether interventions to improve food fussiness could improve zinc status, or whether improvements in zinc status would improve food fussiness in this age group.

**Keywords:** Baby-Led Weaning, complementary feeding, nutrient intake, iron deficiency, zinc deficiency, New Zealand, infants, toddlers, food fussiness
The Candidate was supervised by Associate Professor Anne-Louise Heath from the Department of Human Nutrition, Professor Rachael Taylor from the Department of Medicine, and Emeritus Professor Rosalind Gibson and Professor Samir Samman both from the Department of Human Nutrition.

Associate Professor Anne-Louise Heath and Professor Rachael Taylor were the co-Principal Investigators of the BLISS study and were responsible for securing funding and designing the study along with the study biostatistician (Associate Professor Sheila Williams). The BLISS study was conducted in Dunedin, New Zealand at the University of Otago. Participants of the BLISS study were recruited between November 2012 and March 2014 and data collection was completed in April 2016.

The Candidate was responsible for the following:

Development of blood sample collection methods:

- Developed the blood testing protocols, questionnaires and resources for blood collection.
- Selected and obtained materials used for blood collection.
- Collaborated with the phlebotomist and the study paediatrician to determine the appropriate method of blood sample collection.

Blood sample data collection and laboratory work:

- Contacted all participants prior to their scheduled blood collection visit to determine infant illness and arranged rescheduling of appointments when infants were unwell.
- Conducted all blood sample collection visits, either at the Human Nutrition clinic or as a home visit.
- Conducted all laboratory work associated with blood samples, with assistance from Dr Karl Bailey for plasma zinc analysis, and Ashley Duncan and Michelle Harper for the analysis of inflammatory markers and soluble transferrin receptor.
Liaised with Southern Communities Laboratories Ltd. and delivered all blood samples for the analysis of iron indices.

Entered all blood collection data.

Drafted letters to the participant and the participant’s general practitioner when iron results were outside the relevant reference ranges.

Communicated verbally with participants and general practitioners regarding abnormal iron results.

**BLISS data collection:**

Assisted in the coordination of participant bookings, follow up phone calls, and text reminders.

Conducted approximately one third of the 12-month measurement appointments (n=206 total) for the collection of anthropometric data and questionnaires, including explanation of the weighed diet record.

**Dietary data entry and Kai-culator:**

Assisted in cooking all BLISS intervention recipes for the determination of the cooked weight.

Entered all BLISS intervention recipes into Kai-culator.

Determined the appropriate estimation of breast milk intakes for participants at 12 months of age, using previous literature.

Developed the protocol for checking (accuracy and consistency) weighed diet records.

Checked all 12 month weighed diet records (n=143).

Developed food groups for dietary analysis.

Generated nutrient values (energy, iron, zinc, and selenium) for each food consumed during the BLISS study (n=1682), which were imported into Kai-culator for food group analysis.

Conducted thorough checks of exported dietary data from Kai-culator for inconsistencies, specifically for nutrients and food components of interest: iron, zinc, phytate, and Vitamin C.

**Statistical analysis and writing:**

Conducted data cleaning and coding of all variables.
Prepared results tables to guide analyses for Chapters 4 and 5, and collaborated with the study biostatistician to complete these analyses.

Carried out the statistical analyses in Chapter 6 with the guidance of the study biostatistician.

Prepared the first and subsequent drafts of manuscripts arising from Chapters 4 and 5 (currently in the process of submission).

Conferences and publications:

- Prepared the first and subsequent drafts of the published BLISS study protocol paper.


- Presented results from **Chapter 4** at conferences in New Zealand and Switzerland.


Acknowledgements

Thank you to everyone that has made this PhD journey an unforgettable one. What a team effort this research has been and I really enjoyed working with every one of you during the last three years.

My primary supervisors, Associate Professor Anne-Louise Heath and Professor Rachael Taylor, thank you for believing in me to take on this research project. I really appreciate the time you both gave to supervising me. Your experience, guidance and support has been invaluable, and has helped me to achieve high standards.

Emeritus Professor Rosalind Gibson and Professor Samir Samman, thank you for your supervision, support and expert advice.

My convener, Associate Professor Rachel Brown, thank you for your advice and support.

The BLISS research team – Jenny McArthur-Aitken, Liz Fleming, Brittany Morison, Louise Fangupo, Dr Sara Boucher, Vikki Wood, Rhondda Davies, Dr Ben Wheeler, Professor Barry Taylor, Dr Sonya Cameron. Thank you, you have all been amazing to work with.

Glenna Paterson, you made it look so easy taking blood samples off all of our babies. I was super proud of your success rate, finding the babies tiny veins under mostly chubby arms. Thank you for teaching me how to be a human tourniquet. We made a great team helping parents to feel calm during a potentially anxious time.

My husband (I am still not used to this), Brad, thank you for your never-ending love and emotional support through all my years of study, and for reminding me to enjoy the little things.

My family - mum, dad, Nina, and Aaron, thank you for your love, support and encouragement through all my years of study. I absolutely love spending every Sunday night having dinner with you all. My nephew Quinn, you truly are the best nephew ever. Your smiles and cuddles make everything better. Thank you for being my baby model in this thesis and being so darn cute at it.
Associate Professor Sheila Williams and Dr Jill Haszard, thank you for your advice and support with my statistics. I have learnt so much from you both.

Dr Karl Bailey, Michelle Harper, and Ashley Duncan, thank you for your assistance and expertise in the laboratory.

Liz Fleming and Charlie Blakey, thank you for your help and support with Kalcuator, we definitely challenged the system but I appreciate your efforts and perseverance to make it work.

The Participants of the BLISS study - for whom I absolutely loved working with, thank-you for contributing to this study and being part of this research journey with me and the rest of the BLISS team.

Nicola Hartley, although we are now on other ends of the country, I always enjoy our long skype catch ups. Thank you for your support through all my years of study.

Michelle and Harriet, we make an inseparable trio, when together, always together, your friendship and support are invaluable.

Postgraduate Students, thank you for sharing this experience with me and for your support and friendship.

The University of Otago, thank you for supporting my studies with a PhD scholarship.

Finally, but no means least, thank you to Everyone in the Departments of Human Nutrition and Medicine for your help, kindness, quizzing, coffee chats, and the best morning teas.
# Table of Contents

Abstract ........................................................................................................................................... iii
Preface ................................................................................................................................................ v
Acknowledgements ......................................................................................................................... viii
Table of Contents ............................................................................................................................ x
List of Tables ..................................................................................................................................... xiv
List of Figures ..................................................................................................................................... xvii
Abbreviations ..................................................................................................................................... xviii
Glossary ................................................................................................................................................ xix

1 Introduction ..................................................................................................................................... 1

2 Literature Review ............................................................................................................................ 5

  2.1 Literature aim and search methods .......................................................................................... 5
    2.1.1 Aim of this literature review ............................................................................................. 5
    2.1.2 Search methods .................................................................................................................. 5

  2.2 Complementary feeding approaches ....................................................................................... 7
    2.2.1 What is complementary feeding? ....................................................................................... 7
    2.2.2 Traditional spoon-feeding ................................................................................................. 7
    2.2.3 Baby-Led Weaning ............................................................................................................. 8
    2.2.4 Potential benefits and risks of Baby-Led Weaning ............................................................. 19

  2.3 Determining nutrient intakes in very young children ............................................................... 22
    2.3.1 Dietary assessment methods ............................................................................................ 22
    2.3.2 Methods for estimating breast milk intake ...................................................................... 23
    2.3.3 Determining adequacy of nutrient intakes ....................................................................... 25

  2.4 Iron intakes in very young children ......................................................................................... 27
    2.4.1 Food sources of iron and its absorption modifiers ............................................................ 27
    2.4.2 Iron intakes of very young children in New Zealand ....................................................... 29

  2.5 Iron status of toddlers .............................................................................................................. 40
    2.5.1 Why is the iron status of toddlers important? ................................................................ 40
    2.5.2 Indices for determining iron status .................................................................................... 41
    2.5.3 Inflammation and infection ............................................................................................... 45
    2.5.4 Definitions of stages of iron deficiency ............................................................................. 45
    2.5.5 Iron status of New Zealand toddlers .................................................................................. 48

  2.6 Zinc intakes in very young children ......................................................................................... 49
    2.6.1 Food sources of zinc and its absorption modifiers ............................................................ 49
    2.6.2 Zinc intakes of very young children in New Zealand ........................................................ 51
2.7 Zinc status of toddlers .................................................................................. 56
  2.7.1 Why is the zinc status of toddlers important? .............................................. 56
  2.7.2 Indices for determining zinc status ............................................................. 56
  2.7.3 Biochemical zinc status of New Zealand toddlers ........................................ 58
  2.7.4 Factors associated with biochemical zinc status in very young children ... 59
2.8 Conclusion ........................................................................................................... 86
2.9 Aim and objectives of this thesis ........................................................................ 87
3 Methods ..................................................................................................................... 89
  3.1 Study design and participants .......................................................................... 89
  3.2 Sample size ........................................................................................................ 91
  3.3 Randomisation .................................................................................................... 91
  3.4 Study groups and intervention .......................................................................... 92
    3.4.1 Control ......................................................................................................... 92
    3.4.2 BLISS .......................................................................................................... 92
  3.5 Adherence .......................................................................................................... 97
  3.6 Questionnaire data ............................................................................................. 97
  3.7 Anthropometric assessment .............................................................................. 98
  3.8 Dietary assessment ............................................................................................. 98
  3.9 Dietary analysis .................................................................................................. 99
    3.9.1 Infant milk intake ......................................................................................... 100
    3.9.2 Food group analysis .................................................................................... 101
    3.9.3 Nutrient analysis .......................................................................................... 105
    3.9.4 Analysis of additional food components ...................................................... 106
  3.10 Blood sample collection .................................................................................. 107
  3.11 Biochemical analysis ....................................................................................... 108
    3.11.1 Iron analysis ................................................................................................ 110
    3.11.2 Zinc analysis ................................................................................................ 111
    3.11.3 Analysis of inflammatory markers .............................................................. 111
    3.11.4 Selenium analysis ....................................................................................... 112
  3.12 Adverse events ................................................................................................ 112
  3.13 Statistical analysis ........................................................................................... 112
4 Impact of a baby-led approach to complementary feeding on iron intake and status ................................................................................................................. 113
  4.1 Introduction ....................................................................................................... 115
  4.2 Methods ............................................................................................................. 117
    4.2.1 Data collection ............................................................................................... 117
    4.2.2 Statistical analysis ........................................................................................ 117
4.3 Results .................................................................................................................. 121
  4.3.1 Participant characteristics .............................................................................. 121
  4.3.2 Adherence to a baby-led approach and to delaying the introduction of complementary foods .............................................................................. 124
  4.3.3 Infant milk intakes ....................................................................................... 124
  4.3.4 Intakes of iron and iron absorption modifiers .............................................. 126
  4.3.5 Infant milk intakes and their contribution to iron intakes ............................ 129
  4.3.6 Contribution of complementary foods to iron intakes ................................. 129
  4.3.7 Contribution of intervention foods (red meat and iron-fortified infant cereal) to iron intakes ......................................................................................... 134
  4.3.8 Prevalence of inadequate iron intakes ......................................................... 137
  4.3.9 Biochemical iron status .............................................................................. 137

4.4 Discussion ........................................................................................................... 139

5 Impact of a baby-led approach to complementary feeding on zinc intake and status ............................................................................................................. 151
  5.1 Introduction ....................................................................................................... 153
  5.2 Methods ............................................................................................................ 154
    5.2.1 Data collection ............................................................................................ 154
    5.2.2 Statistical analysis .................................................................................... 154
  5.3 Results ............................................................................................................... 157
    5.3.1 Participant characteristics ....................................................................... 157
    5.3.2 Adherence to a baby-led approach and to delaying the introduction of complementary foods ................................................................. 161
    5.3.3 Infant milk intakes .................................................................................... 161
    5.3.4 Zinc and phytate intakes ........................................................................ 162
    5.3.5 Complementary food sources of zinc ....................................................... 165
    5.3.6 Biochemical zinc status ........................................................................... 168
  5.4 Discussion ........................................................................................................... 169

6 Potential predictors of zinc status in toddlers: a cross-sectional analysis ................................................................................................................................. 175
  6.1 Introduction ....................................................................................................... 177
  6.2 Methods ............................................................................................................ 178
    6.2.1 Data collection ........................................................................................... 178
    6.2.2 Statistical analysis .................................................................................... 178
  6.3 Results ............................................................................................................... 181
    6.3.1 Maternal and infant characteristics at baseline ........................................ 181
    6.3.2 Participant characteristics at 12 months of age ...................................... 183
    6.3.3 Univariate associations between 'predictor' variables and plasma zinc concentrations ............................................................................................... 185
6.3.4 Multivariate regression analysis of ‘predictors’ of plasma zinc concentrations at 12 months of age ........................................................................................................ 188
6.4 Discussion ....................................................................................................................... 191
7 Conclusions and recommendations ................................................................................... 201
  7.1 Summary and conclusions ............................................................................................. 201
  7.2 Recommendations for future research .......................................................................... 202
  7.3 Recommendations for clinical use ................................................................................. 204
References ............................................................................................................................... 207
Appendices ............................................................................................................................. 227
  Appendix A: BLISS baseline questionnaire ................................................................. 229
  Appendix B: BLISS in a nutshell resource ........................................................................ 237
  Appendix C: BLISS laminated everyday foods to offer resources ................................. 241
  Appendix D: Breastfeeding and solids questionnaire .................................................. 245
  Appendix E: 12-month questionnaire ................................................................................. 257
  Appendix F: BLISS weighed three-day diet record ....................................................... 281
  Appendix G: Weighed diet record calculation sheet and entry protocol ....................... 293
  Appendix H: Protocol for checking weighed diet records ............................................. 309
  Appendix I: Estimation of breast milk intakes at 12 months of age ............................ 317
  Appendix J: Contribution of individual foods to food groups ....................................... 321
  Appendix K: Protocol for arranging blood collection appointment ............................ 327
  Appendix L: Blood test appointment card ....................................................................... 337
  Appendix M: Pre-blood test instruction sheet ................................................................. 341
  Appendix N: Protocol for reminder blood test phone call and illness questionnaire ...... 347
  Appendix O: Protocol for blood sample collection and analysis .................................... 353
  Appendix P: Checklist for during blood collection appointment ................................... 365
  Appendix Q: Zinc questionnaire for during blood collection appointment .................. 369
  Appendix R: Protocol for communicating abnormal blood results .............................. 373
  Appendix S: Analysis of methodological factors known to affect plasma zinc concentrations ................................................................. 381
List of Tables

**Table 2.1** Search methods and terms used within this literature review .......... 5

**Table 2.2** Guideline for texture progression of infants following a traditional spoon-feeding approach to complementary feeding, adapted from the New Zealand Ministry of Health (2008) ................................................... 8

**Table 2.3** Baby-Led Weaning studies to date .................................................................. 10

**Table 2.4** Summary of the most common factors shown to modify non-haem iron absorption from the World Health Organization (2001) and the Institute of Medicine (2002) ........................................................................... 29

**Table 2.5** Studies assessing the iron intakes and/or status of very young children in New Zealand (from year 1995 onwards) ........................................... 31

**Table 2.6** Summary of dietary factors shown to modify zinc absorption, and dietary factors that may modify zinc absorption .................................................................. 51

**Table 2.7** Studies assessing the zinc intakes and/or status of very young children in New Zealand (from year 2000 onwards) ........................................... 53

**Table 2.8** Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards) ........................................................................... 66

**Table 3.1** Individual foods included in each food group for food group analysis .................................................................................................................. 102

**Table 3.2** An example recipe showing the proportion of each food group and contribution to nutrient intakes for a 100 g portion of cooked cheesy carrot and courgette slice ........................................................................... 105

**Table 3.3** Criteria used to determine the stage of iron deficiency ........................................ 111

**Table 4.1** Characteristics of participants who provided intake data at 7 \( (n=162) \) and/or 12 \( (n=143) \) months of age or biochemical data at 12 \( (n=119) \) months of age ........................................................................... 120

**Table 4.2** Comparisons of those included (provided either intake or status data) and not included (provided neither intake nor status data) in this data set ........................................................................... 122

**Table 4.3** Milk consumers at 7 and 12 months of age ........................................... 125

**Table 4.4** Intake of iron and key iron absorption modifiers at 7 months of age from complementary foods and infant milks ........................................... 127
Table 4.5 Intake of iron and key iron absorption modifiers at 12 months of age from complementary foods and infant milks.......................... 128

Table 4.6 Iron from complementary foods at 7 months of age (consumers and non-consumers) ................................................................. 131

Table 4.7 Iron from complementary foods at 12 months of age (consumers and non-consumers) ................................................................. 132

Table 4.8 Number of consumers of each food group at 7 and 12 months of age ............................................................................................ 133

Table 4.9 Dietary sources of iron for consumers only at 7 months of age (complementary foods and infant milks) ........................................ 135

Table 4.10 Dietary sources of iron for consumers only at 12 months of age (complementary foods and infant milks) ........................................ 136

Table 4.11 Iron status indicators and categories at 12 months of age .......... 138

Table 5.1 Characteristics of participants who provided intake data at 7 (n=162) and/or 12 (n=143) months of age or biochemical data at 12 (n=119) months of age ........................................................................ 158

Table 5.2 Comparisons of those included (provided either intake or status data) and not included (provided neither intake nor status data) in this data set ........................................................................................................ 159

Table 5.3 Intake of zinc and phytate at 7 months of age from complementary foods and infant milks ............................................................ 163

Table 5.4 Intake of zinc and phytate at 12 months of age from complementary foods and infant milks .................................................................. 164

Table 5.5 Contribution of food groups (other than infant milks) to zinc intake at 7 months of age ........................................................................ 166

Table 5.6 Contribution of food groups (other than infant milks) to zinc intake at 12 months of age ........................................................................ 167

Table 5.7 Plasma zinc concentrations at 12 months of age ................. 168

Table 6.1 Maternal and infant baseline characteristics of participants who provided biochemical zinc data at 12 months of age ............ 182

Table 6.2 Characteristics of participants who provided biochemical zinc data at 12 months of age ................................................................ 184

Table 6.3 Univariate associations between ‘predictor’ variables and plasma zinc concentration (μmol/L) at 12 months of age .................. 186
Table 6.4 Pearson’s correlation (r) analysis to identify potential collinearity between modifiable ‘predictor’ variables ................................................................. 188

Table 6.5 Frequency of significant associations between ‘predictor’ variables and plasma zinc concentration in a stepwise bootstrap analysis (n=104) ........................................................................................................................................ 189

Table 6.6 Multivariate model of ‘predictors’ of plasma zinc concentrations at 12 months of age (n=103) .................................................................................................................. 190
List of Figures

Figure 1.1 Flow diagram demonstrating the three key outcomes of the BLISS study and the contribution of this thesis .................................................................................. 3

Figure 2.1 The effect on common biochemical measures of iron status through the stages of iron deficiency, adapted from Suominen et al. (1998) ........................................................................................................................................ 46

Figure 3.1 CONSORT diagram of the BLISS study participants, as relevant to the analysis of iron and zinc intake and status..............................................................90

Figure 3.2 Intervention and outcome measures at specific time points (as published in Daniels et al. (2015)) ......................................................................................... 96

Figure 3.3 Blood sample processing flowchart ........................................................................... 109

Figure 6.1 Plasma zinc concentrations (μmol/L) of participants (n=115) ...... 183

Figure 6.2 Plausible pathways in the association between food fussiness and zinc status .................................................................................................................. 193
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGP</td>
<td>$\alpha_1$-acid glycoprotein</td>
</tr>
<tr>
<td>BLISS</td>
<td>Baby-Led Introduction to Solids</td>
</tr>
<tr>
<td>BLW</td>
<td>Baby-Led Weaning</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated average requirement</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>IBCLC</td>
<td>International Board Certified Lactation Consultant</td>
</tr>
<tr>
<td>IZiNCG</td>
<td>International Zinc Nutrition Consultative Group</td>
</tr>
<tr>
<td>LMC</td>
<td>Lead maternity carer</td>
</tr>
<tr>
<td>MFP</td>
<td>‘Meat, fish, poultry’</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>NZDep</td>
<td>New Zealand Index of Deprivation</td>
</tr>
<tr>
<td>NZEO</td>
<td>New Zealand European and others</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended dietary intake</td>
</tr>
<tr>
<td>SCL</td>
<td>Southern Community Laboratories Ltd.</td>
</tr>
<tr>
<td>SES</td>
<td>Socio-economic status</td>
</tr>
<tr>
<td>sTfR</td>
<td>Soluble transferrin receptor</td>
</tr>
<tr>
<td>UL</td>
<td>Upper intake level</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>WDR</td>
<td>Weighed three-day diet record</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Glossary

Definitions used within this thesis:

- **Infants:** 0-11.9 months
- **Toddlers:** 12-24 months (or 1-2 years)
- **Very early childhood:** Infants and toddlers (as above)
- **Very young children:** Infants and toddlers (as above)
- **Pre-school children:** 2-4 years
- **School children:** ≥5 years

The World Bank (2016) was used to define the income level of countries throughout this thesis.
1 Introduction

Traditionally, parents introduce complementary foods to their infant by spoon-feeding them puréed food; often starting with a fortified infant cereal or a single fruit or vegetable. Over time, greater texture is introduced so that by the time the infant is 12 months of age, they are generally eating what the rest of the family eats, including a reasonable proportion of finger foods (Ministry of Health, 2008; US Department of Agriculture, 2009; National Health and Medical Research Council, 2012). An alternative approach, known as Baby-Led Weaning (BLW), is increasing in popularity in New Zealand (Cameron et al., 2013), the United Kingdom (Brown et al., 2011a), Canada (D’Andrea et al., 2016), Australia (Cichero, 2016), and the United States (Beal, 2016). In BLW, infants feed themselves all of their food from the start of the complementary feeding period (Rapley et al., 2008). It has been suggested that this self-feeding may help infants maintain the energy self-regulation they demonstrate while they are exclusively milk fed (Rapley et al., 2008). There are several proposed advantages of BLW, including a potential lower risk of obesity, better diet quality, less fussy eating, and more advanced motor skills (Rapley et al., 2008; Cameron et al., 2012b; D’Andrea et al., 2016). However, some concerns have also been expressed by health professionals, namely the potential for an increased risk of iron and zinc deficiency, growth faltering, and choking (Cameron et al., 2012b; D’Andrea et al., 2016).

Because foods must be in a form that the infant can pick up and feed themselves, BLW has the potential to increase the risk of iron and zinc deficiency if the majority of first foods offered to the infant are foods low in these nutrients, such as fruits and vegetables (Ministry of Health, 2010). This could also be problematic if whole foods such as meat which are high in bioavailable iron (Lombardi-Boccia et al., 2002) and zinc (Brown et al., 2004)
are not offered due to parental concerns about choking (Cameron et al., 2012b; D’Andrea et al., 2016). However, very little data exist which have examined iron and zinc in very young children. A recent observational study reported that infants following BLW had mean iron intakes which were less than half those of infants following more traditional spoon-feeding, and mean zinc intakes which were also significantly lower by around 20% (Morison et al., 2016). However, the impact of these lower intakes of important nutrients on biochemical iron and zinc status in very young children has not been examined in that (Morison et al., 2016), or any other study to date.

Recommended intakes of iron and zinc are often not consumed during the complementary feeding period (World Health Organization, 2004b), and may be an even greater issue when following a baby-led approach compared with more traditional spoon-feeding practices, as outlined above. Poor iron intakes may lead to iron deficiency, a common nutritional deficiency globally, and potentially iron deficiency anaemia, which is associated with delays in cognitive function that may not be reversible (Domellöf et al., 2014). On the other hand, poor zinc status may have implications for immune function (Shankar et al., 1998; Fraker et al., 2000), and growth (Nissensohn et al., 2016). In addition, we currently do not know whether there may be potentially modifiable factors which could improve zinc status and therefore prevent zinc deficiency in very young children in high-income countries such as New Zealand.

To date, no study has examined both the iron and zinc intakes, and the iron and zinc status of infants following a baby-led approach to complementary feeding. Nor have there been any studies to determine potentially modifiable ‘predictors’ of zinc status in high-income countries.

The overall aim of the Baby-led Introduction to SolidS (BLISS) randomised controlled trial was to determine whether a modified version of BLW prevents young children from becoming overweight, without increasing their risk of iron deficiency, growth faltering (Taylor et al., 2017), and choking (Fangupo et al., 2016). The aim of this thesis was to determine the impact of this version of BLW modified to prevent iron deficiency, on iron and zinc intakes
and status (Figure 1.1), and to determine potentially modifiable ‘predictors’ of zinc status in toddlers. The specific objectives were to:

1. Determine the iron intake (at 7 and 12 months of age) and iron status (at 12 months of age) of infants following BLISS compared with those of infants following traditional spoon-feeding (Chapter 4).
2. Determine the zinc intake (at 7 and 12 months of age) and zinc status (at 12 months of age) of infants following BLISS compared with those of infants following traditional spoon-feeding (Chapter 5).
3. Examine associations between biochemical, dietary, and other variables, and plasma zinc concentration, and to determine potentially modifiable ‘predictors’ of zinc status at 12 months of age (Chapter 6).

Figure 1.1 Flow diagram demonstrating the three key outcomes of the BLISS study and the contribution of this thesis
A summary of the chapters included in this thesis are outlined below:

**Chapter 2** presents a summary of the literature on complementary feeding approaches, including BLW. An evaluation of the methods for assessing iron and zinc intake and status is also presented, including previous findings on the intakes and status of very young children in New Zealand. Lastly, this chapter describes factors which have been shown to be associated with zinc status in children.

**Chapter 3** describes the BLISS study methods. The BLISS intervention is described, as well as the methods of data collection. However, statistical analyses relevant to each chapter are reported with their respective results.

** Chapters 4 and 5** address objectives one and two of this thesis. The statistical analyses and results regarding the assessment of the iron and zinc intakes (at 7 and 12 months of age) and the iron and zinc status (12 months of age only) of infants following BLISS compared with infants following traditional spoon-feeding are reported. The findings are discussed in relation to previous study findings.

**Chapter 6** addresses objective three of this thesis. The specific statistical analyses and results investigating modifiable ‘predictors’ of zinc status at 12 months of age are reported. The findings are discussed in relation to previous study findings.

**Chapter 7** concludes the thesis and provides recommendations for future research, and for clinical use of the results of this thesis.
2 Literature Review

2.1 Literature aim and search methods

2.1.1 Aim of this literature review
Complementary feeding approaches that are used currently (traditional spoon-feeding and Baby-Led Weaning) are reviewed in this chapter with a focus on Baby-Led Weaning (BLW). As health professionals have expressed concerns about the potential increased risk of iron and zinc deficiency when following a baby-led approach to complementary feeding, this literature review also evaluates methods for assessing the intake and status of iron and zinc, and the current iron and zinc intakes and status of very young children in New Zealand (NZ) during the complementary feeding period (around 6-12 months of age).

2.1.2 Search methods
Literature searches were conducted to 31 March 2017 and were performed using Medline (Ovid MEDLINE® 1946 to Present with Daily Updates) and ScienceDirect. Searches were limited to articles published in English. Table 2.1 outlines the search methods and key terms used. Relevant references were also obtained from the reference lists of the articles retrieved.

Table 2.1 Search methods and terms used within this literature review

<table>
<thead>
<tr>
<th>Search terms for complementary feeding related studies (Section 2.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) complementary feeding.mp</td>
</tr>
<tr>
<td>2) infant feeding.mp</td>
</tr>
<tr>
<td>3) baby-led.mp</td>
</tr>
<tr>
<td>4) BLW.mp</td>
</tr>
</tbody>
</table>
### Search terms for dietary assessment and nutrient intake methods (Section 2.3)

1) dietary assessment.mp
2) breast milk.mp
3) milk, human/
4) test-weighing.mp
5) stable isotope.mp
6) usual intake.mp
7) (2) and (3) and [{4} or {5}]

### Search terms for iron related studies (Sections 2.4 and 2.5)

1) iron intake.mp
2) iron status.mp
3) infants.mp
4) infant/
5) toddlers.mp
6) New Zealand/
7) iron/
8) inflammation/
9) (1) and [{3} and (4) or (5) or (6)]
10) (2) and [{3} and (4) or (5) or (6)]
11) (8) and (9)

### Search terms for zinc related studies (Sections 2.6 and 2.7)

1) zinc intake.mp
2) zinc status.mp
3) infants.mp
4) infant/
5) toddlers.mp
6) New Zealand/
7) inflammation/
8) association.mp
9) (1) and [{3} and (4) or (5) or (6)]
10) (2) and [{3} and (4) or (5) or (6) or (7) or (8)]

**Bold** indicates terms searched. Relevant abstracts were screened for inclusion in this literature review.
2.2 Complementary feeding approaches

2.2.1 What is complementary feeding?
Complementary feeding is defined by the World Health Organization (WHO) as “the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed, along with breast milk” (World Health Organization, 2004b), but it is most commonly used to refer to the introduction of solid foods. The most recent guideline for the timing of introducing complementary foods is to begin when the infant is 6 months (180 days) of age (World Health Organization, 2003), although this has been interpreted as ‘around’ 6 months of age by the NZ Ministry of Health (2008). By this age complementary foods need to ‘complement’ the energy and nutrients provided by breast milk to ensure appropriate growth and development of the infant (Ministry of Health, 2008). There are two main approaches to complementary feeding which are described below: traditional spoon-feeding, and BLW.

2.2.2 Traditional spoon-feeding
Traditionally, infants are spoon-fed at the start of complementary feeding and then over time, infants are gradually introduced to solid foods with increased texture and variety (World Health Organization, 2004b). This method of complementary feeding is used as a guideline for many national health agencies including the NZ Ministry of Health (Ministry of Health, 2008) and other government agencies globally (National Health and Medical Research Council, 2012; US Department of Agriculture, 2009). The texture progression occurs at different age stages, when the infant is considered to be at the appropriate developmental stage to progress to a new texture (World Health Organization, 2004b) (Table 2.2). By approximately 12 months of age (although this will vary between children), the infant should be eating the same foods as the rest of the family (Ministry of Health, 2008).
Table 2.2 Guideline for texture progression of infants following a traditional spoon-feeding approach to complementary feeding, adapted from the New Zealand Ministry of Health (2008)

<table>
<thead>
<tr>
<th>Approximate age (months)</th>
<th>Appropriate texture of food</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 6</td>
<td>Liquid</td>
</tr>
<tr>
<td>6 – 7</td>
<td>Purée</td>
</tr>
<tr>
<td>7 – 8</td>
<td>Mashed including some finger food¹</td>
</tr>
<tr>
<td>8 – 12</td>
<td>Chopped</td>
</tr>
<tr>
<td>12 – 24</td>
<td>Family foods²</td>
</tr>
</tbody>
</table>

¹Finger foods are foods that can be picked up by the child and eaten “with the fingers”
²Family foods are foods that are eaten by the rest of the family, in the form that they are eaten by the rest of the family

2.2.3 Baby-Led Weaning

BLW differs from traditional spoon-feeding in that the use of purées and spoon-feeding is generally bypassed, with infants being encouraged to feed themselves all of their foods from the beginning of the complementary feeding period (Rapley, 2011). This means that foods offered to the infant need to be finger foods that they can pick up and feed themselves, as young infants do not have the ability to feed themselves liquid or semi-liquid foods using utensils.

While most countries recommend that finger foods are introduced early in the complementary feeding period (World Health Organization, 2004b; Ministry of Health, 2008; National Health and Medical Research Council, 2012; Health Canada, 2014), they generally only represent a small component of the diet, particularly in the first few months of complementary feeding. By contrast, in BLW, parents choose a range of foods to offer their infant and then the infant decides which of the foods to eat, how much, and at what pace they will eat (Rapley, 2011). The key features of BLW include (Rapley, 2011; Cameron et al., 2012a):

- **Milk feeding** – ideally the infant will be exclusively breastfed until 6 months of age, although it is acknowledged that some infants will be formula fed.

When complementary feeding starts (once the infant is ready at around 6
months of age) the infant continues to receive milk feeds (breast milk or infant formula) on demand.

- **Baby-led** – the infant self-feeds from the beginning of the complementary feeding period. Generally, puréed foods are not consumed because of their liquid consistency, which makes it difficult to self-feed. Some families may offer the child utensils so that they can feed themselves purées or foods with a thin consistency (e.g., yoghurt and custard) but this is unlikely in the first few months for developmental reasons, as it is difficult for an infant to operate spoons and other utensils accurately.

- **Family foods** – the infant is offered the same foods as the family but in the form of finger foods that are large enough for them to pick up. These pieces can get smaller with increasing developmental age as long as parents are aware of the dietary guidelines regarding foods that pose an increased risk of choking.

- **Family meals** – the family eats together at mealtimes.

Despite BLW growing in popularity in many regions around the world (Cameron et al., 2013; Brown & Lee, 2011a; D’Andrea et al., 2016; Beal, 2016; Cichero, 2016), very little research has directly examined the advantages and disadvantages of this alternative approach to introducing complementary foods to infants (Table 2.3). The majority of research currently conducted in this area is observational. Overall, the research to date relies on the experiences of parents, their beliefs and often self-reports of following BLW, and has not explored the potential risks and benefits associated with this alternative approach to complementary feeding. There is also disagreement about what constitutes BLW, as there is no formal definition for it.
### Table 2.3 Baby-Led Weaning studies to date

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
</table>
| Brown et al., (2011a)      | United Kingdom       | \(n = 655\) Mothers with infants aged 6-12 months. Recruited through local nurseries, community centers, baby groups in the local city, and online parenting websites. | Cross-sectional. Non-validated online questionnaire. BLW definition = spoon-feeding or purée feeding \(\leq 10\%\) of the time. | Parents using BLW were more likely to be highly educated, have high-level occupation, be married and have breastfed their infant. BLW associated with: later introduction of complementary foods, less likely to offer purées as a first food, greater participation at meal times, greater exposure to family foods and fewer meals offered over the day. First food was more likely to be fruit or vegetable for BLW, and baby-rice for those spoon-feeding. Maternal anxiety concerning nutrient status and mess was associated with spoon-feeding. But use of spoon-feeding was associated with reduced confidence at meal times compared with BLW. | • Non-validated questionnaires.  
• Some questions were retrospective - relied on recall.  
• Sample recruitment bias due to targeting the BLW community including use of online BLW websites. |
| Brown et al., (2011b) (same sample as previous study (Brown et al., 2011a)) | United Kingdom       | \(n = 652\) Mothers with infants aged 6-12 months. Recruited through local nurseries, community centers and internet parenting sites. | Cross-sectional. Online validated questionnaire. BLW definition = spoon-feeding or purée feeding \(\leq 10\%\) of the time. | Parents using BLW were more likely to be highly educated, have high-level occupation and less likely to have returned to work. BLW associated with lower levels of restriction, pressure to eat, monitoring and concern for infant weight. No association between weaning style and infant weight (birth weight, weight at 6 months and current weight). Mothers following BLW thought their infant was significantly larger in first 6 months than standard weaning (SW) mothers. | • Potential sample recruitment bias.  
• Self-reported weight. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Sample Size</th>
<th>Design</th>
<th>BLW Definition</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright et al.</td>
<td>United Kingdom</td>
<td>$n = 510$ (all data)</td>
<td>Prospective cohort.</td>
<td>BLW definition = not defined</td>
<td>40% of participants offered finger foods before 6 months, 90% had been offered finger foods before 8 months. Most common foods offered when offering finger foods were bread (31-39%), or rusks or biscuits (32-43%). Fruit and vegetables (11-20%), confectionary (2-5%), and meat (1-2%) were offered less frequently.</td>
<td></td>
</tr>
<tr>
<td>Townsend et al.</td>
<td>United Kingdom</td>
<td>$n = 155$</td>
<td>Case-control.</td>
<td>BLW definition = self-defined</td>
<td>Those following BLW had a significantly greater preference for carbohydrates than those following SW. Those following SW had a greater preference for sweet foods compared with those following BLW. BLW was associated with lower BMI. More BLW children classified as underweight compared with SW and more SW were children classified as obese compared with BLW.</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Country</td>
<td>Participants</td>
<td>Design/methods</td>
<td>Main findings</td>
<td>Study limitations</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>----------</td>
<td>--------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Rowan et al.</td>
<td>United States</td>
<td>$n = 10$ Mothers with infants approaching 6 months. Recruited through social media (Facebook).</td>
<td>Cross-sectional. Questionnaires and 3DD diaries of infant’s diet at baseline (5-6 months) and 3 months following BLW initiation (8-9 months). BLW definition = participants read Gill Rapley’s book and planned to feed their infant using BLW.</td>
<td>Only parental intake and not infant intake were assessed. Higher intakes of undesirable nutrients such as saturated fat, sodium and sugar were reported and inadequate total energy and folate. Only the types of foods offered to infants were reported, which suggested a wide variety of foods were consumed. Meals were offered to infants on average 3.5 times per day and 3 meal occasions were with a parent. Infants continued to be milk fed on average 5 times per day (breast or formula fed). No significant change in the parental diet was seen before and after initiating BLW with their infant. On average 57% of the food consumed at a meal was the same between the parent and infant.</td>
<td>• Small sample size. • Sample recruitment bias due to targeting the BLW community including use of online BLW websites. • Non-validated questionnaires • Under-reporting and recall bias is common with the 3DD method of assessment. • Very little information regarding the infant diet. • Did not assess infant intakes of key nutrients, such as iron.</td>
<td></td>
</tr>
</tbody>
</table>
Cameron et al. (2012b) New Zealand  

$n = 51$ (all data)  
Health care professionals ($n=31$). Mothers who had used BLW ($n=20$). Recruited by word of mouth (parenting groups), email ‘snowballing’ or newspaper advertisements.  

Cross-sectional. Semi-structured interview. BLW definition = self-defined.  

Focused on attitudes, knowledge and experiences of BLW.  

Knowledge - Health care professionals:  
Many (42%) had heard about BLW from family, friends or colleagues.  

Knowledge - Mothers:  
Most defined BLW as having 3 main meals offering finger sized foods and allowing the child to be in control – no spoon-feeding.  

Attitudes - Health care professionals:  
Believe BLW will be beneficial for the family and child with the sharing of meal times, encouragement of healthy dietary patterns, may encourage better appetite control and had developmental advantages. They also had concerns for the risk of choking, potential risk of growth faltering, poor iron status, mess and food waste. Some thought that BLW could encourage eating beyond their needs with poor food choices, and could increase parent anxiety about infant weight.  

Attitudes - Mothers:  
Chose BLW for lifestyle reasons and it seemed logical, less expensive and less time consuming than making puréed food. Advantages were: less meal preparation, reduced mealtime stress, healthier eating behaviours. Most had no concerns; those listed included: appropriateness of foods to offer, iron intake and mess.  

- Small sample size.  
- Qualitative data – relying on participants’ knowledge and experiences only.  
- Potential sample recruitment bias.
### Table 2.3 (continued) Baby-Led Weaning studies to date

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown et al. (2013a)</td>
<td>United Kingdom</td>
<td>n = 36 Mothers with infants aged 12-18 months. Recruited through online advertisements on BLW websites.</td>
<td>Cross-sectional. Semi-structured interviews (attitudes and experiences). BLW definition = spoon or purée feeding ≤10% of the time.</td>
<td><strong>Positive reports:</strong> Timing of introduction of complementary foods coincided with developmental readiness. Family meals were adapted to be suitable for the infant – cooking style, use of salt, sugar and fat. The family sat together at meals. Offered a larger variety of foods, which is thought to improve the infant’s approach to food later in life. Feeding style was low in control – amount consumed and type of food. <strong>Negative reports:</strong> Initial concerns regarding the amount the infant was eating; however, this became less of a problem over time. Variety of nutrients may not be enough. Other challenges: mess, food waste, anxiety around gagging and choking. <strong>Overall:</strong> This method made sense to participants. Considered to be simple, convenient, low cost, less stressful and fits well with family lifestyle. Eating foods in their natural form rather than processed.</td>
<td>• Small sample size. • Sample recruitment bias due to participants recruited from specific BLW websites. • Mothers had previously followed BLW with their infant - relied on recall of experiences.</td>
</tr>
<tr>
<td>Cameron et al. (2013)</td>
<td>New Zealand</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n = 199$ Mothers with infants aged 6-12 months. Recruited through newspaper advertisements.</td>
<td>Cross-sectional. Online questionnaire. BLW definition = adherent BLW (tried BLW and infants mostly self-fed) or self-identified BLW (tried BLW but spoon feed 50% of the time).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most (70%) followed a parent-led approach to complementary feeding, 21% self-identified BLW and 19% adherent BLW. Adherent BLW participants were more likely to have family foods, as well as having these from the beginning of complementary feeding, and were less likely to have commercially produced foods. Compared with the self-identified BLW and parent-led participants, adherent BLW children were not offered iron-fortified infant cereal as their first food. Choking was reported in 33% of all participants, mostly reported after consuming whole foods. Mothers who had followed BLW would recommend it and almost 60% of mothers would recommend a combination of BLW and SW. Of the parent-led feeders, 46% would try BLW with another child. Parent concerns regarding BLW were: choking (55.3%), not consuming enough (44.2%), not enough motor skills to self-feed (27.6%).</td>
<td>• Non-validated questionnaire. • Some questions were retrospective - relied on recall. • Potential recruitment bias.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Country</td>
<td>Participants</td>
<td>Design/methods</td>
<td>Main findings</td>
<td>Study limitations</td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------------</td>
<td>-------------------------------</td>
<td>-----------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Brown et al. (2013b)</td>
<td>United Kingdom</td>
<td>n = 298</td>
<td>Longitudinal, cross-sectional. Validated questionnaires. BLW definition = spoon-feeding or purée feeding ≤10% of the time.</td>
<td>Mothers following BLW had significantly lower concern for child weight, pressure to eat, restriction and monitoring. No difference between BLW and SW in perceived responsibility. BLW infants were more satiety responsive (better appetite control), less food responsive and less food fussy than SW. Breastfed infants were more satiety-responsive and less food fussy than non-breastfed infants. Introducing complementary foods earlier was associated with increased fussiness. A higher level of fussiness was associated with maternal concern for infant weight. Child weight was lower in BLW infants with 5.4% defined as underweight compared with 2.5% in SW infants. Infants following SW were significantly heavier than those following BLW. Four main themes reported on: Trust – participants reported trusting their baby’s ability to choose the timing, type and amount of food consumed. Parental control – participants reported high level of monitoring and control over feeding process, especially regarding delaying introduction of foods until 6 months. Role of milk – breast milk was considered to be very important to participants in first year of life due to bonding and nutrient intake. BLW approach – some parents deviated from the rules of BLW to avoid mess and assist when the baby was unable to self-feed.</td>
<td>• Sample recruitment bias due to targeting the BLW community including use of online BLW sites. • Self-reported weight. • Small number of participants classified as underweight (SW: n=3, BLW: n=9). • Some questions were retrospective - relied on recall.</td>
</tr>
<tr>
<td>Arden et al. (2014)</td>
<td>United Kingdom</td>
<td>n = 15</td>
<td>Cross-sectional. Five semi-structured email questionnaires sent to each participant. BLW definition = self-defined.</td>
<td></td>
<td>• Small sample size. • Non-validated questionnaires. • Sample recruitment bias due to participants recruited from specific BLW websites.</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Sample Size</td>
<td>Data Collection</td>
<td>Outcome</td>
<td>Potential Limitations</td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>-------------</td>
<td>-----------------</td>
<td>---------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Moore et al. (2014)</td>
<td>United Kingdom</td>
<td>n = 3607 Parents of infants of unknown age. Recruited through parent support organisations, online forums and advertisements.</td>
<td>Cross-sectional. Online validated questionnaire. BLW definition = self-defined.</td>
<td>Knowledge of weaning guidelines was associated with later introduction of complementary foods. A ‘baby-led’ or ‘finger-feeding’ approach predicted probability of late weaning (at or greater than 26 weeks).</td>
<td>- Potential recruitment bias.</td>
</tr>
<tr>
<td>Cameron et al. (2015)</td>
<td>New Zealand</td>
<td>n = 23 (all data) BLISS (n=14), BLW (n=9). Families with infants aged 5 months. Recruited through local free newspaper.</td>
<td>Pilot intervention. Interviews weekly for 12 weeks and 3-day WDR or 3x 24 hr iron questionnaire. Intervention group received 2 visits, resources and on call support. BLW definition = participants chose between BLW or BLISS (modified version of BLW).</td>
<td>Reported on the 3 main concerns of BLW – potential risk of iron deficiency, potential risk of growth faltering and potential risk of choking. Iron – grams of red meat offered to infants in the BLISS group was significantly higher than the BLW group. BLISS infants were offered a wider variety and more serves of iron containing foods than the BLW group. Energy – No difference in energy intake between the groups. Choking – No difference in reports of choking episodes between the groups. Consumption of high-choking risk foods was lower in BLISS compared with BLW.</td>
<td>- Small sample size. - High participant burden with weekly interviews for 12 weeks. - Iron intake data only collected for a small subsample (n=10). - WDR only collected from a small subsample (n=8). - No control, SW group. - Participants were not randomly assigned to groups but self-selected.</td>
</tr>
<tr>
<td>Reference</td>
<td>Country</td>
<td>Participants</td>
<td>Design/methods</td>
<td>Main findings</td>
<td>Study limitations</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------</td>
<td>------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>D’Andrea et al. (2016)</td>
<td>Canada</td>
<td>n = 98 (all data) HCP (n=33). Mothers who had used BLW (n=65). Recruited through BLW Facebook page and health authorities.</td>
<td>Cross-sectional. Online questionnaires. BLW definition = self-defined.</td>
<td>Mothers chose BLW as it “made sense” and “felt natural”. Most mothers sourced information regarding BLW from online groups and friends/family. 97% of mothers offered fruits and vegetables as first foods and 57% offered meat as a second food. 45% of mothers were concerned about the risk of choking, but only 5% indicated a choking episode. HCP’s mostly sourced information regarding BLW through other HCP’s. HCP’s reported a greater concern for risk of choking, inadequate energy and iron intake.</td>
<td>• Non-validated questionnaires. • Sample recruitment bias due to participants recruited from specific BLW sites. • Some participants answered retrospectively.</td>
</tr>
<tr>
<td>Morison et al. (2016)</td>
<td>New Zealand</td>
<td>n = 51 (all data) Age- and sex-matched infants 6-8 months. BLW (n=25), TSF (n=26). Mothers recruited through three previous studies.</td>
<td>Cross-sectional. Questionnaires and either a 3-day or 1-day WDR. BLW definition = based on question asked after completing WDR “what approach to infant feeding were you using’ – ‘spoon-feeding’ or ‘BLW’.</td>
<td>Those following BLW breastfed for longer, introduced complementary foods later (including iron-fortified infant cereal) and were less likely to introduce complementary foods before 6 months compared to TSF. No difference between BLW and TSF in the number of high-choking risk foods consumed. BLW infants consumed significantly less protein and CHO (% of total kJ), fibre, iron, zinc, vitamin C, vitamin B12, and calcium than TSF infants. However, BLW infants consumed significantly more fat (g and % total kJ), and saturated fat (g and % total kJ) than TSF infants. BLW infants were significantly more likely to have 2 of the 3 main meals (lunch and dinner) with the family, as well as consuming the same foods, prepared the same as the rest of the family compared with TSF infants.</td>
<td>• Small sample size. • Subjectively asked parents if they were following ‘spoon-feeding’ or ‘BLW’. • Potential recruitment bias. • No infant biochemical status data.</td>
</tr>
</tbody>
</table>

Abbreviations: 3DD, three-day diet diary; BLW, Baby-Led Weaning; CHO, carbohydrate; en, energy; HCP, health care professional; SW, standard weaning (spoon-fed/parent-led); TSF, traditional spoon-feeding; WDR, weighed diet record.
2.2.4 Potential benefits and risks of Baby-Led Weaning

There are a number of proposed benefits and risks associated with BLW, although research directly examining whether these exist is lacking (Rapley et al., 2008). In brief, some potential benefits include:

A potentially lower risk of obesity as a result of: better energy self-regulation, better diet quality, and favourable effects on parental feeding practices (Rapley et al., 2008). However, to date only two observational studies have investigated rates of obesity in infants following BLW with conflicting results (Townsend et al., 2012; Brown et al., 2011b). Brown et al. (2011b) found no association between the complementary feeding method (BLW or spoon-feeding) and infant weight as reported by parents at 6 months. In contrast, Townsend et al. (2012) reported a significantly lower Body Mass Index (BMI) and prevalence of obesity in children at 20-78 months who had followed BLW compared with traditional spoon-feeding. However, both studies are limited by the use of self-reported weight, which is particularly problematic in this rapidly growing age group. Proposed better diet quality and healthy eating behaviours have not been formally assessed, however, the parental perception is that BLW encourages healthier eating and decreases picky/fussy eating (Cameron et al., 2012b; D’Andrea et al., 2016). A further proposed benefit is more advanced motor skills (Carruth et al., 2002), as both parents and health care professionals perceive BLW will promote fine and oral motor skills (Cameron et al., 2012b; D’Andrea et al., 2016).

There are also some potential risks that have been raised including: a potential increased risk of iron deficiency, growth faltering, and choking (Cameron et al., 2012b; D’Andrea et al., 2016). Health professionals have expressed concern regarding growth faltering, based on the assumption that not all infants will have the motor skills, or motivation, to feed themselves the amount of food they require, and that many of the first foods offered will be low in energy (Cameron et al., 2012b). To date, only two cross-sectional studies appear to have examined growth in infants following BLW, both finding a higher
prevalence of underweight in infants following BLW compared with spoon-feeding (Townsend et al., 2012; Brown et al., 2013b). However, both of these studies used self-reported weight, and only a small proportion of the infants were considered underweight (1-3%). In terms of choking, Cameron et al. (2013) found that 33% of mothers who participated in an online survey considered that their infant had had a choking episode, which was most often (74%) after the consumption of whole food. However, there was no difference in the risk of choking between those self-fed and spoon-fed ($p=0.57$) (Cameron et al., 2013). Results from the BLISS pilot study (Cameron et al., 2015), and choking outcomes from the BLISS study (Fangupo et al., 2016) showed no difference between the BLISS and Control groups in the number of choking occasions reported. However, it should be noted that many infants were consuming high-choking risk foods whether they were self-fed or spoon-fed and that BLISS was a version of BLW modified to reduce choking risk (Fangupo et al., 2016; Morison et al., 2016).

No study to date has assessed the risk of iron and zinc deficiencies in infants following BLW and only one study has assessed nutrient intakes in infants following BLW compared with traditional spoon-feeding (Morison et al., 2016). In that small study ($n=51$), infants following BLW had on average 59% ($p<0.001$) lower intakes of iron and 21% ($p=0.001$) lower intakes of zinc compared with traditionally spoon-fed infants. However, there was no measure of biochemical status in the study by Morison et al. (2016), and therefore it is not known whether any increased risk of iron and zinc deficiency occurred from the lower intakes in infants fed following the BLW approach to complementary feeding.

In conclusion, several proposed benefits and risks of BLW have been reported. However, the majority of current BLW studies are limited by their observational nature, use of self-reported data, reliance on retrospective experiences, small sample sizes, and recruitment bias (Table 2.3). Therefore, before this alternative approach to traditional complementary feeding can be recommended, further research is required. Specifically, randomised controlled trials are needed to determine whether the proposed benefits and risks
reported in the current observational data are actually caused by a baby-led approach.

**Components of Sections 2.2.3 and 2.2.4 are published in the BLISS study protocol paper**

2.3 Determining nutrient intakes in very young children

2.3.1 Dietary assessment methods

To estimate the nutritional adequacy of a population or a group of very young children, the use of a dietary assessment method that collects information on ‘usual’ food intake is needed (Burrows et al., 2010). There are several different methods that can be used to estimate dietary intakes such as: 24-hour diet recalls, diet histories, food frequency questionnaires, estimated diet records, and weighed diet records. Limitations of these methods include that they are highly sensitive to measurement error by both the participant (e.g., incorrect estimations, over- or under-reporting) and the researcher (e.g., calculations and assumptions when entering data using nutrient composition databases) (Gibson, 2005a).

There are also particular limitations when assessing dietary intakes in very young children that need to be taken into account. These include the fact that they need to be completed on behalf of the infant by a caregiver, which is complicated further in that often infants are in the care of several people (e.g., grandparents, child care), and that illness can influence the dietary intakes of infants. Both these factors can introduce measurement error (Institute of Medicine, 2000). Limitations can be reduced by thoroughly explaining to participants how to complete the dietary assessment method, and by using protocols to standardise the process of data handling for researchers, including the use of accurate food composition data (Gibson, 2005a).

The weighed diet record is widely considered to provide the best estimate of dietary intakes in very young children (0.5-4 years) (Burrows et al., 2010). However, particular limitations of this method include: an increased burden on the participant because they have to weigh all foods consumed, and changes to food consumption patterns on recording days due to ease of recording, and beliefs as to what is considered healthy or unhealthy (Burrows et al., 2010). Therefore, nutrient inadequacy of a population may be overestimated
if foods (and therefore nutrients) are under-reported (Institute of Medicine, 2000).

Other factors that need to be taken into account when determining dietary intakes of infants are: how to measure/estimate breast milk intakes, and determining the adequacy of nutrient intakes.

2.3.2 Methods for estimating breast milk intake

It is difficult to accurately measure breast milk intakes; however, breast milk is an important contributor to nutrient intakes of infants. There are two methods for measuring breast milk intakes which are used: ‘test weighing’ and the deuterium oxide stable isotope method (International Atomic Energy Agency, 2010).

The ‘test weighing’ method is more commonly used than the deuterium oxide method (Dewey et al., 1984; Nommsen et al., 1991; Dewey et al., 1991a). This method involves weighing the infant before and after every breastfeed and then calculating the difference in weight to determine the amount of breast milk consumed (Savenije et al., 2006). It has been proposed that this method may not reflect normal feeding practices because the inconvenience of the process may lead to the under-estimation of actual milk intakes (Savenije et al., 2006). However, Dewey et al. (1991b) reported no differences in the frequency of feeding when they asked participants to record how often they breastfed their child over 7 days compared to the frequency obtained during 4 days of ‘test weighing’. Nor did they see a decline in breast milk volumes consumed over the 4 days of ‘test weighing’ (Dewey et al., 1991b), suggesting this concern is not warranted. Measurement of breast milk intakes via this method may produce slight underestimates due to losses during feeding, particularly insensible water losses (i.e. water loss from the skin and respiratory tract), but these can be corrected for (Scanlon et al., 2002).

The deuterium oxide stable isotope method is considered the gold standard method for measuring breast milk intakes, however, is less often used, most probably because of methodological constraints and cost (International
Atomic Energy Agency, 2010). In this method, the amount of milk consumed by
the infant is assessed over a 14-day period using deuterium oxide labeled water
(International Atomic Energy Agency, 2010). The labeled water is consumed by
the mother and the amount of deuterium oxide remaining in the mother and
passed onto the infant is measured (usually via saliva samples) over several
days (International Atomic Energy Agency, 2010). Although this method has
been used to assess breast milk intakes in infants less than 12 months of age (da
Costa et al., 2010; Lennox et al., 2011), no studies were found in high-income
countries assessing estimated breast milk intakes of toddlers (from 12 months
of age).

A small study comparing the two methods above found that
measurements of breast milk intake were not significantly different, and
therefore concluded that both of these methods are appropriate for the
estimation of breast milk intakes (Butte et al., 1988).

When actual breast milk intake cannot be measured using ‘test weighing’
or deuterium oxide stable isotope methods, authors have used estimates from
previously published literature (Skinner et al., 1997; Ponza et al., 2004; Butte et
al., 2010; Briefel et al., 2010). In these studies, either a total average breast milk
intake per day (e.g., 448 g/day (Dewey et al., 1991a)) or an average quantity per
breast feed (e.g., 89 mL/feed (Butte et al., 2010)) is used for infants of a given
age. When a total average breast milk amount is used, the amount of other
infant milk (e.g., infant formula) consumed can be subtracted from the
estimated breast milk volume to give an estimate of the total quantity of breast
milk consumed (Skinner et al., 1997; Ponza et al., 2004; Butte et al., 2010;
Briefel et al., 2010). Using previously published estimates is cost effective,
however, measured intakes are variable (which is not taken into account with
this approach), and after 6 months of age estimating breast milk intake becomes
complicated by the introduction of solid food, which likely results in a wider
distribution of actual breast milk intakes. For large studies, and studies
collecting dietary data at multiple time points, particularly those for which
breast milk intake is not a primary outcome, estimation is often necessary
because the respondent and researcher burden of ‘test weighing’ and the deuterium oxide method makes them impractical.

2.3.3 Determining adequacy of nutrient intakes

The nutrient intakes of a group should not be directly compared to the recommended dietary allowance, or an equivalent measure, to determine the nutrient adequacy of a group (Gibson, 2005b). This approach could severely overestimate the proportion at risk of inadequate intakes (Gibson, 2005b). Instead, reliable statistical methods such as the full probability approach or the Estimated Average Requirement (EAR) cut-point method should be used to determine the prevalence of inadequate nutrient intakes of groups (Murphy et al., 2006; Gibson et al., 2008).

To be able to determine the proportion of a particular group at risk of inadequate nutrient (e.g., iron or zinc) intakes, information on the ‘usual’ (long term) intake of the group is required (Murphy et al., 2006). Dietary assessment data (from dietary assessment methods described in Section 2.3.1) collected from participants provides dietary intake data that can be used to determine ‘usual’ intakes. Statistical methods should be used to mathematically transform data to remove intra-individual variability, providing a ‘usual’ intake distribution (Souverein et al., 2011). This ‘usual’ intake distribution can then be used to estimate the proportion of a group at risk of inadequate nutrient intakes using one of two methods (the full probability approach or the EAR cut-point method).

The **full probability approach** should be used for nutrients with a skewed requirement distribution (e.g., iron) (Gibson et al., 2008). In this approach, the ‘usual’ nutrient intake data for each individual is associated with a pre-determined risk-probability range for that particular age and sex group, for example those defined by the Institute of Medicine (2002). The percentage of individuals falling within each range of intake, is then multiplied by the associated probability of risk of inadequacy for that intake level so that the total
of all the weighted risk, represents the total probability of inadequate nutrient intakes for the group of interest (Institute of Medicine, 2002).

The **EAR cut-point method** can be used for nutrients which have a symmetrical requirement distribution (e.g., zinc) (Gibson et al., 2008). Using the ‘usual’ nutrient intake data of a group, the proportion of individuals with a ‘usual’ intake below the EAR for the specific nutrient gives us the prevalence of inadequate intakes for the group. For zinc, the population risk of zinc deficiency is considered to be elevated when the overall probability of inadequate intakes is greater than 25% (Brown et al., 2004).
2.4 Iron intakes in very young children

2.4.1 Food sources of iron and its absorption modifiers

The amount of iron transferred through breast milk is sufficient to maintain adequate growth and development until 6 months of age (World Health Organization, 2001). However, after this time the iron concentration in breast milk is reduced and the intake of complementary foods high in iron becomes important in ensuring iron intakes are adequate (World Health Organization, 2001). Infants who are fed infant formula have higher intakes of iron, as iron is added to formulas in large quantities to counteract the poor bioavailability of this form of iron, compared with breast milk (Fairweather-Tait, 1989).

There are two forms of iron (haem and non-haem) which are absorbed differently in the body (Institute of Medicine, 2002). Haem iron is highly bioavailable and found in animal products (beef, lamb, poultry, seafood etc.), whereas non-haem iron is less bioavailable and found in various amounts in most foods, including: meat, legumes, cereals, nuts, seeds, vegetables, and iron-fortified foods. The amount of non-haem iron absorbed from an individual meal differs depending on other food components within the meal, which can modify (enhance or inhibit) the absorption of iron by around 1-40% (Institute of Medicine, 2002; World Health Organization, 2001).

It has long been recognised that flesh foods such as meat, fish, and poultry, as well as ascorbic acid, improve iron bioavailability by enhancing non-haem iron absorption (World Health Organization, 2001; Institute of Medicine, 2002). By contrast, phytate, and polyphenols (e.g., tannins in tea), and perhaps calcium are known to inhibit iron absorption (Table 2.4) (World Health Organization, 2001; Institute of Medicine, 2002).

Phytate is particularly high in cereal products, and cereal products are commonly consumed as complementary foods for infants. Because cereals are so frequently consumed, and are dry and relatively low in fat, they are good vehicles for iron fortification. Although the phytate content will inhibit the absorption of some of the added iron (World Health Organization, 2001), this
can be partially offset by the addition of ascorbic acid, which improves iron bioavailability by reducing the impact of phytate on non-haem iron absorption (Fairweather-Tait, 1989; Davidsson et al., 2000). In addition, phytate is particularly high in wholegrain cereals and these are not usually given to infants. (Ministry of Health, 2008). Although limited evidence is available regarding the potential influence of phytate on iron absorption in infants (Gibson et al., 2010), in one study it was reported that the iron absorption of infants was similar to adults after consumption of formula containing phytate and formula containing added ascorbic acid (Hurrell et al., 1999), suggesting similar effects of enhancers and inhibitors on iron absorption in adults and infants.

High consumption of cow’s milk (≥500 mL per day) in very young children has been shown to be associated with lower ferritin concentrations (depleted iron stores) (Daly et al., 1996; Soh et al., 2004; Gunnarsson et al., 2004; Uijterschout et al., 2014; Domellöf et al., 2014; Bramhagen, 1999). While the high calcium content in food and beverages such as milk are known to reduce total iron absorption from a single meal (Hallberg et al., 1991), this appears to affect iron absorption in the short term only, as there appears to be no evidence of an impact of these high calcium intakes on iron status (Lönnerdal, 2010). The effect of high consumption of cow’s milk on iron status in very young children is more likely due to the fact that cow’s milk is low in iron and high intakes may displace other complementary foods in the diet which are important sources of iron and other nutrients (Ministry of Health, 2008; Ziegler, 2011), but also that high cow’s milk intake is associated with increased gastrointestinal blood loss in infants (Wilson et al., 1974; Ziegler, 1990). The NZ Ministry of Health and other health agencies do not recommend infants consume cow’s milk as a drink until after 12 months of age (Ministry of Health, 2008; American Academy of Pediatrics, 1992).
Table 2.4 Summary of the most common factors shown to modify non-haem iron absorption from the World Health Organization (2001) and the Institute of Medicine (2002)

<table>
<thead>
<tr>
<th>Intake modifier</th>
<th>Enhances non-haem iron absorption</th>
<th>Inhibits non-haem iron absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Meat, Fish, Poultry' factor</td>
<td>Ascorbic acid (Vitamin C)</td>
<td>Phytate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polyphenols, such as tannins and other iron-binding phenolic compounds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcium, high intakes of milk and dairy products may affect both haem and non-haem absorption</td>
</tr>
</tbody>
</table>

2.4.2 Iron intakes of very young children in New Zealand

Previous research has reported that mean iron intakes are between 1.6 and 8.4 mg per day for NZ infants (Wham, 1996; Soh et al., 2001; Heath et al., 2002; Wall et al., 2008; Morison et al., 2016) and between 4.3 and 6.3 mg per day for NZ toddlers (Soh et al., 2001; Heath et al., 2002; Wall et al., 2008; Szymlek-Gay et al., 2009). The wide range of intakes observed is probably a function of limitations inherent in these data, in particular, a) the fact that different methods were used to collect dietary data, b) low energy reporting may have been more of an issue for some studies than others - those studies with iron intakes on the lower end also had overall lower mean energy intakes compared with the estimated energy requirements, and c) most of the studies were not in a representative sample of children (see Table 2.5).

Only one of these studies reported the risk of inadequate iron intakes - using the EAR cut-point method (described in Section 2.2.3) to calculate the proportion of very young children with ‘usual’ intakes below the United Kingdom EAR for iron. In total 15% of infants and 66% of toddlers in that study were considered to be at risk of inadequate iron intakes (Soh et al., 2001). These limited data would suggest that iron intakes in infants are likely to be adequate, whereas inadequate iron intakes may be more of an issue in toddlers. However, further studies are required to confirm this result.
Table 2.5 describes the studies reported in Sections 2.4.2 and 2.5.5 and provides more detail including the findings regarding the iron intakes and status of very young children in NZ, and the limitations of each study.
Table 2.5 Studies assessing the iron intakes and/or status of very young children in New Zealand (from year 1995 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wham (1996)</td>
<td>n = 53</td>
<td>Cross-sectional.</td>
<td></td>
<td>• Small sample size.</td>
</tr>
<tr>
<td></td>
<td>Very young children aged 9-24 months. Recruited through paediatrician and GP’s as an opportunistic sample.</td>
<td>Intake: 24h recall and diet history. Status: Non-fasting venous sample. ID defined as SF &lt;10 μg/L. Anaemia defined as Hb &lt;110 g/L.</td>
<td>Iron intake Mean (SD), mg/day: 9-12 months: 6.2 (4.4). 13-16 months: 5.1 (3.4). 17-20 months: 4.5 (3.1). 21-24 months: 4.8 (1.9). Those with higher iron intakes were mostly formula fed and majority of their intakes came from formula and commercial baby food. Iron status Median SF (μg/L): 23. Median Hb (g/L): 119.5. ID: 13% (n=7). IDA: 19% (n=10).</td>
<td>• Use of 24h diet recall and diet history may have resulted in recall bias - leading to under- or over-estimation of intake. • Did not use the above data to determine ‘usual’ intakes. • Single reference values used for determining ID (SF) and IDA (Hb). • Did not report if any inflammation/infection was present, which may have resulted in an underestimation of the prevalence of ID and IDA.</td>
</tr>
</tbody>
</table>
## Table 2.5 (continued)  
Studies assessing the iron intakes and/or status of very young children in New Zealand (from year 1995 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soh et al.</td>
<td>n = 323</td>
<td>Cross-sectional. General questionnaire.</td>
<td>Iron intake</td>
<td>• Excluded infants who were BF from dietary analysis as the amount of BM could not be quantified (n=75).</td>
</tr>
<tr>
<td>(2001)</td>
<td>Very young children aged 6-24 months. Recruit as randomly selected sample – cluster sampling.</td>
<td>Intake: 3-day WDRs. Probability approach to determine prevalence of inadequate intake (proportion of ‘usual’ intakes below the EAR). Status: Non-fasting venous sample. Infants defined as &lt;12 months. Toddlers defined as &gt;12 months. Cut offs used: SF &lt;10 μg/L. Inflammation: CRP ≥10 mg/L.</td>
<td>Median (quartiles), mg/day: &lt;12 months girls: 8.6 (5.7, 9.5). &lt;12 months boys: 8.3 (6.8, 11.5). ≥12 months girls: 4.8 (3.4, 6.6). ≥12 months boys: 4.4 (3.4, 6.6). 15% infants and 66% toddlers at risk of inadequate iron intakes. No significant difference in iron intakes based on season. For infants (non-BF) - 60% of iron intakes came from iron-fortified infant formula, followed by commercial infant foods (8%), and fruit and vegetables (7%). For toddlers (non-BF) - 31% of iron intakes came from cereals, followed by fruit and vegetables (15%), and MFP (10%). Only 16% toddlers consumed iron-fortified infant cereal. Iron status Low SF: 15% (n=37) all children &lt;8% infants and 20% toddlers had low SF.</td>
<td>• Blood samples were not obtained from all infants (n=263, 81%). • Only reported SF (iron stores) results in this paper as further results regarding iron status were reported in the later paper (Soh et al., 2004).</td>
</tr>
<tr>
<td>Heath et al. (2002)</td>
<td>n = 74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very young children 9-24 months. Mothers of newborn babies were found through birth notices and the local newspaper and contacted by telephone.</td>
<td>Longitudinal study. Demographic information at initial visit. Weekly telephone calls to determine information on infant feeding. <strong>Intake:</strong> Estimated 24h diet record (prior to each monthly visit). 3DDR at 12, 18 and 24 months. <strong>Status:</strong> Capillary blood sample (heel prick). Anaemia defined as low Hb. IDA defined as anaemia and low MCV. IDE defined as low MCV and elevated ZPP. Cut offs used: Hb &lt;110 g/L MCV &lt;77 fL ZPP &gt;80 μg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Iron intake</strong></td>
<td><strong>Iron status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (25th, 75th percentiles), mg/day: 9 months: 7.0 (3.7, 10.7). 12 months: 4.3 (3.2, 7.1). Median iron intakes were lower than the estimated requirements at all ages. Vitamin C and calcium intakes appear adequate at all ages.</td>
<td>ID (IDE) without anaemia prevalence: 19% (n=6) at 9 months, 22% (n=7) at 12 months, 13% (n=4) at 24 months. IDA prevalence: 7% (n=4) at 9, 12 and 18 months, 0% (n=0) at 24 months.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Did not calculate BM intake - estimated BM intake based on previous published work. • Did not report if any inflammation/infection was present. • Socioeconomically advantaged group compared to the general population of NZ. • Use of 24h diet recall may have resulted in memory lapses of what was actually consumed. For some time points only 24h recall data was used which is not a good reflection of ‘usual’ intake. • Used estimated rather than weighed diet records which may lead to under- or over-estimation of intake. • Did not assess SF as a measure of iron stores. • Compared median intakes to the EAR which is misleading and should not be used.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.5 (continued) Studies assessing the iron intakes and/or status of very young children in New Zealand (from year 1995 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant et al.</td>
<td>(n = 391) Very young children aged 8-23 months. Very young children who were hospitalised for acute illness (respiratory, non-localised febrile illnesses, gastroenteritis, acute surgical, or orthopaedic). Excluded if Hb &lt; 70 g/L.</td>
<td>Prospective study. Closed-ended questionnaire to assess feeding patterns. <strong>Intake:</strong> Not reported. <strong>Status:</strong> Full blood count. Stages of ID defined based on: SF, iron saturation, and RDW (2 of 3 abnormal). Cut offs used: Hb &lt;110 g/L, SF &lt;10 (\mu)g/L, Iron saturation &lt;10%, RDW &gt;14.5%, Inflammation: CRP &gt;6 mg/L.</td>
<td>Iron intake Not reported. <strong>Iron status</strong> Non-ID: 25% ((n=98)). ID without anaemia: 19% ((n=73)). IDA: 56% ((n=220)). Stratified groups based on CRP – proportion with abnormal indices remained. Prevalence of IDA higher in those of Pacific ethnicity, those diagnosed with pneumonia, mother restricted meat intake during pregnancy, had more than three children in the house, and were currently being breastfed (after adjusting for CRP). Prevalence of ID was higher in those of Pacific ethnicity, and those who were drinking tea. Lower prevalence of ID in those diagnosed with gastroenteritis, or pneumonia (after adjusting for CRP).</td>
<td>- Sample of very young children with acute illness – makes it difficult to compare incidence of IDA with other studies in healthy infants as disease states and inflammation affect these indices. - CRP was not obtained from all infants ((n=313), 80%). - Used a questionnaire to assess household income/expenditure and dietary intake patterns – potential recall bias. - Did not assess dietary intake using usual methods, e.g., FFQ or WDR.</td>
</tr>
</tbody>
</table>
Soh et al. (2004) (same sample as Soh et al. (2001))

- **n = 323**
- Very young children aged 6-24 months.
- Recruited as randomly selected sample – cluster sampling.

Cross-sectional.
- General questionnaire (self-administered).
- Weight and length.
- **Intake:** 3-day WDRs.
- Probability approach to determine prevalence of inadequate intake (proportion of ‘usual’ intakes below the EAR).

**Status:** Non-fasting venous sample.
- Stages of ID defined based on: SF, MCV, and/or ZPP (at least 2 of 3 abnormal). IDA was ID and low Hb.
- Cut offs used:
  - Hb <110 g/L
  - SF <10 μg/L and <12 μg/L
  - ZPP >70 μmol/mol haem
  - MCV <73 fl

**Iron intake**
- Not reported.

**Iron status**
- Participants with normal CRP (SF <12 μg/L):
  - Depleted iron stores: 8% at 6-11.9 months, 23% at 12-24 months.
  - ID without anaemia: 4% at 6-11.9 months, 6% at 12-24 months.
  - IDA: 7% at 6-11.9 months, 3% at 12-24 months.
- Mean (SD) 6-11.9 months:
  - Hb (g/L): 111 (9).
  - MCV fl: 77.4 (3.3).
  - ZPP (μmol/mol haem): 50.0 (16.6).
  - SF (μg/L): 22.9 (2.0).
- Mean (SD) 12-24 months:
  - Hb (g/L): 116 (10).
  - MCV fl: 77.8 (3.8).
  - ZPP (μmol/mol haem): 47.0 (15.7).
  - SF (μg/L): 16.0 (1.8).

- Sex, ethnicity, and birth weight were positively associated with SF. Age, and weight-for-age were negatively associated with SF.

- Blood samples were not obtained from all infants (n=263, 81%).
- This paper reports mostly biochemical data, whereas previous paper: Soh et al. (2001) mostly reports the iron intake data.
Table 2.5 (continued) Studies assessing the iron intakes and/or status of very young children in New Zealand (from year 1995 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant et al. (2007) (same sample as Wall et al. (2008))</td>
<td>n = 416 Very young children aged 6-23 months. Ethnically stratified random sample recruited through random residential address by cluster sampling. No exclusion criteria.</td>
<td>Cross-sectional. Weight and length. <strong>Intake:</strong> Not reported. <strong>Status:</strong> Venous blood sample. Full blood count. ID defined as 2 or more of: iron saturation, MCV, and RDW (below cut off). IDA defined as ID and Hb below cut off. Cut offs used: Hb &lt;110 g/L SF &lt;10 μg/L Iron saturation &lt;10% MCV &lt;73 fl RDW &gt;14% Inflammation: CRP &gt;4 mg/L (grouped to compare with those with normal CRP ≤4 mg/L).</td>
<td><strong>Iron intake</strong> Not reported. <strong>Iron status</strong> ID without anaemia (normal CRP): 14% (n=49). IDA (normal CRP): 6% (n=23). Median (5th, 95th percentile): Hb (g/L): 119 (100, 134). Ferritin (μg/L): 20 (4,57). Iron saturation (%): 16.2 (5.4, 31.6); MCV (fl): 77 (67, 83); RDW (%): 14.1 (12.6, 16.5).</td>
<td>• Did not have sufficient statistical power (required 450 children). • Did not report if blood sample was collected fasted or non-fasted. • Did not state how they collected information on: milk feeding, or any dietary intake. • Included the use of RDW for determining iron status, the cut off for children is not very sensitive (Gibson, 2005c).</td>
</tr>
</tbody>
</table>

Univariate analysis: Those of Māori, Pacific or other ethnicities other than NZ European were at greater risk of ID (although not specifically powered to examine this). Continued BF past 6 months, not receiving infant formula, and consuming cow’s milk were associated with increased risk of ID. Infants consuming homemade baby food compared with commercial baby food were at increased risk of ID. Receiving iron supplements before 12 months decreased risk of ID. Infants with 2 or more siblings were at increased risk of ID compared with singletons. Risk of ID did not vary with household deprivation.

Multivariate analysis: only BMI ≥18.5 kg/m², and not receiving infant formula were significant risk factors for ID.
Wall et al. (2008) (same sample as Grant et al. (2007))

- **n = 307**
- Very young children aged 6-23 months.
- Recruited as randomly selected residential addresses - cluster sampling.

**Cross-sectional.**

- Weight and length.

**Intake:** 2-day WDRs.

**Status:** Venous blood sample.

- ID defined as 2 or more SF, transferrin saturation, and MCV (below cut offs). IDA defined as ID and Hb below cut off.

- Cut offs used:
  - Iron saturation <10%
  - SF <10 μg/L
  - Hb <110 g/L
  - MCV <73 fl

**Inflammation:** CRP >4 mg/L (excluded participants with raised CRP from the analysis).

**Iron intake**

- Median (25<sup>th</sup> & 75<sup>th</sup> percentiles) 6-11 months:
  - Energy (kJ/kg): 325 (242, 389).
  - Fe (mg/day): 8.3 (4.7, 10.8).
  - Vitamin C (mg/day): 90 (42, 125).

- Median (25<sup>th</sup> & 75<sup>th</sup> percentiles) 12-23 months:
  - Energy (kJ/kg): 337 (284, 394).
  - Fe (mg/day): 6.3 (4.3, 8.8).
  - Vitamin C (mg/day): 67 (41, 119).

**Dietary sources of iron differed by age.** BM and milk formulas contributed the most (58%) iron at 6-11 months, whereas cereals contributed the most (41%) at 12-23 months.

**Iron status**

- Total iron intake was positively associated with SF at both age points.
- SF increased with increased birth weight (6-11 months), intake of iron from breast milk/milk formulas (6-23 months), and Vitamin C intake (12-23 months).
- SF decreased with increased Ca intake (6-11 months), and iron intake from non-human or non-formula milk (12-23 months).

- Ethnic differences occurred in the associations between SF and dietary intakes.

- **Assessment of dietary intake based on 2-day WDR may not be reflective of ‘usual’ intake – although energy intakes were similar to that estimated for this age.**

- **WDR were not completed by all (n=247, 80%).**

- **Although associations were reported between dietary factors and SF – the authors did not state the mean SF in this paper, which would be useful to know.**

- **Only univariate associations were reported and therefore this did not take into account potential confounding factors in the associations with ethnicity.**

The previous paper reported no association between ethnicity and ID in the multivariate model (Grant et al. 2007).
Table 2.5 (continued) Studies assessing the iron intakes and/or status of very young children in New Zealand (from year 1995 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Szymlek-Gay et al. (2009)</td>
<td>n = 225</td>
<td>Randomised controlled trial. Randomised into: meat group (n=90), fortified toddler milk group (n=45), or placebo group (n=90). Demographic questionnaire (self-administered). Weight and length. Intake: 3-day WDR at baseline, week 4 and week 18 of the intervention. Status: Non-fasting venous blood samples at baseline and post intervention. Delayed 2 weeks if unwell. Depleted iron stores defined as SF ≤12 μg/L. ID defined as 2 or more: SF, MCV, and ZPP (below cut offs). IDA defined as ID and Hb below cut off. Cut offs used: SF ≤12 μg/L, Hb &lt;110 g/L, MCV ≤73 fL, ZPP ≥70 μmol/mol haem Inflammation: CRP ≥10 mg/L</td>
<td></td>
<td>• Due to smaller number of participants in the fortified milk group there was less power to detect effects on the iron status of this group (required 90 per group). • Excluded infants who were BF from dietary analysis, as the amount of BM consumed could not be quantified. • Only a figure describes the proportion of infants with depleted iron stores, ID and IDA – actual proportions not presented. • Did not assess changes in IDA and anaemia as these participants were excluded from the analyses.</td>
</tr>
</tbody>
</table>

Iron intake
Geometric mean (95% CI) at baseline, mg/day:
Control group: 4.8 (4.3, 5.3), meat group: 4.4 (4.1, 4.9), fortified milk group: 4.2 (3.7, 4.7).
Iron status
Geometric mean (95% CI) at baseline:
SF (μg/L): 21.7 (19.7, 23.9).
Hb (g/L) - Control group: 119 (118, 119), meat group: 119 (117, 119), fortified milk group: 120 (119, 120).
Transferrin receptor (mg/L) - Control group: 6.8 (6.5, 7.1), meat group: 6.9 (6.5, 7.2), fortified milk group: 6.6 (6.2, 7.0).
Body iron (mg/kg) - Control group: 2.8 (2.3, 3.3), meat group: 2.1 (1.5, 2.7), fortified milk group: 2.5 (1.6, 3.4).
Post intervention SF increased significantly in the fortified milk group, did not change in the red meat group and tended to decrease in the Control group.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 51 (all data)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-</td>
<td></td>
<td>Geometric mean (95% CI), mg/day:</td>
</tr>
<tr>
<td>matched infants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-8months.</td>
<td></td>
<td>Full BLW: 1.6 (1.2, 2.1).</td>
</tr>
<tr>
<td>BLW (n=25), TSF</td>
<td></td>
<td>TSF: 3.6 (2.7, 4.9).</td>
</tr>
<tr>
<td>(n=26).</td>
<td></td>
<td>Iron intakes were significantly lower in BLW</td>
</tr>
<tr>
<td>Mothers recruited</td>
<td></td>
<td>compared with TSF group.</td>
</tr>
<tr>
<td>through three</td>
<td></td>
<td>Iron status</td>
</tr>
<tr>
<td>previous studies.</td>
<td></td>
<td>Not reported.</td>
</tr>
</tbody>
</table>

**Intake:** Questionnaires and either a 3-day or 1-day WDR. BLW definition = based on question asked after completing WDR “what approach to infant feeding were you using’ – ‘spoon-feeding’ or ‘BLW’.

**Status:** Not reported.

- Small sample size.
- Potential recruitment bias.
- No infant biochemical status data.

**Abbreviations:** BF, breast fed; BLW, Baby-Led Weaning; BM, breast milk; Ca, calcium; CRP, C-reactive protein; EAR, estimated average requirement; Fe, iron; FFQ, food frequency questionnaire; GP, general practitioner; Hb, haemoglobin; ID, iron deficiency; IDA, iron deficiency anaemia; IDE, iron deficiency erythropoiesis; MCV, mean cell volume; MFP, ‘meat, fish, poultry’; NZ, New Zealand; RDW, red cell distribution width; SF, serum ferritin; TSF, traditional spoon-feeding; WDR, weighed diet record; UK, United Kingdom; ZPP, zinc protoporphyrin; 3DDR, three-day diet record.
2.5 Iron status of toddlers

2.5.1 Why is the iron status of toddlers important?

The prevalence of iron deficiency is high among very young children worldwide and it is the most common nutritional deficiency, affecting infants motor and cognitive development (World Health Organization, 2001; Domellöf et al., 2014). The prevalence of iron deficiency appears to increase with: low intake of 'high-iron’ foods (Soh et al., 2001; Domellöf et al., 2014), low birth weight (Iannotti et al., 2006; Berglund et al., 2010), low socioeconomic status (Male et al., 2001; Grant et al., 2003; Park et al., 2009) and high consumption of cow's milk (≥500 mL per day) (Daly et al., 1996; Soh et al., 2004; Gunnarsson et al., 2004; Uijterschout et al., 2014; Domellöf et al., 2014; Bramhagen, 1999).

Very young children are at high risk of iron deficiency due to their high growth rate at this age and increasing demand for iron at a time when breast milk no longer provides sufficient amounts (Domellöf et al., 2014). Of particular concern is that iron deficiency anaemia is thought to have long lasting and potentially irreversible negative effects on brain development (Beard, 2008). Although recent meta-analyses agree that iron supplementation reduces the risk of iron deficiency and iron deficiency anaemia in very young children, they appear inconclusive regarding the effect this has on infant development (Pasricha et al., 2013; Thompson et al., 2013). However, these meta-analyses are limited due to the lack of randomised controlled trials available, confounding due to socio-economic factors, and the difficulty in assessing development in children.

It appears that iron deficiency can be prevented not only by supplementation but also by using a food based approach - ensuring infants and toddlers consume sufficient 'high-iron' foods (Domellöf et al., 2014), including the consumption of red meat (Szymlek-Gay et al., 2009) and iron-fortified infant cereal (Walter et al., 1993). The use of foods may be preferred due to the
unknown risks supplementation may pose to otherwise healthy young children (Iannotti et al., 2006).

2.5.2 Indices for determining iron status

The measurement of iron status is complex, and a number of biochemical indices are used as there is no single laboratory test for determining iron status (World Health Organization, 2001). This creates difficulty when comparing iron status across populations as studies use different biochemical indices and cut offs for determining iron status (Table 2.5). The most common indices (haemoglobin, ferritin, soluble transferrin receptor, and body iron) reported are described below.

**Haemoglobin** is the molecule in red blood cells that is responsible for carrying oxygen (Gibson, 2005c). When haemoglobin concentrations fall below the reference range for a given age, anaemia is identified. Many factors affect haemoglobin concentrations including: biological variation (with values higher in the morning than in the afternoon), age, sex, and race (Gibson, 2005c). One key limitation of the use of haemoglobin is that it does not differentiate between anaemia caused by iron deficiency and anaemia due to other causes. So haemoglobin alone cannot be used to assess the iron status of an individual (Gibson, 2005c). There is also controversy regarding the cut off for determining anaemia. Clinically, at an individual level, Southern Community Laboratories Ltd. (SCL, clinical laboratory for Dunedin, NZ) recommend an acceptable haemoglobin concentration of between 105-140 g/L for children aged 6 months to 2 years (Southern Community Laboratories Ltd, 2014). This agrees well with the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) which also suggests a cut off of 105 g/L should be used for children aged 6-24 months (Domellöf et al., 2014). The WHO recommend a slightly higher cut off, indicating that a value of <110 g/L should be used for defining anaemia in a population of children aged 6 months to 5 years (World Health Organization, 2001). However, this value was extrapolated from older age
groups, and could potentially lead to overestimation of rates of anaemia (Gibson, 2005c).

**Serum/plasma ferritin** is the biochemical index most reflective of body iron stores (Gibson, 2005c). Ferritin concentrations change with age and tend to be higher at birth and through adulthood but lowest during childhood (Archer et al., 2015). There are also known sex differences with males having lower ferritin concentrations during infancy and females having lower concentrations during adolescence and into adulthood, which is reflective of differences in overall iron status between males and females (Gibson, 2005c). Ferritin is also affected by ethnicity but only minimally by diurnal variation (Gibson, 2005c). The main limitation of ferritin is that it is a positive acute phase protein – meaning that in response to inflammation, plasma concentrations are artificially elevated (Domellöf et al., 2014) (see Section 2.5.3). Therefore, ferritin is best used in the absence of inflammation, although ferritin concentrations may be statistically adjusted for inflammation (Thurnham et al., 2010). As with haemoglobin, there is controversy regarding the cut off used to determine low ferritin. The WHO state that ferritin concentrations below 12 μg/L indicate storage iron deficiency for children under 5 years of age (World Health Organization, 2001). Domellöf et al. (2014) broadly suggest <10-12 μg/L should be used for defining low ferritin in infants aged 6-24 months, which are values commonly used (Table 2.5). However, at the clinical level, SCL suggests a cut off of <15 μg/L for use in very young children and children (Southern Community Laboratories Ltd., 2012), a value that has been used in some studies in very young children (Oti-Boateng et al., 1998; Capozzi et al., 2010). The term ‘plasma’ ferritin will be used to refer to plasma or serum ferritin in this literature review, unless reporting results from studies which specifically assessed serum ferritin.

**Soluble transferrin receptor** (sTfR), also known as serum transferrin receptor, which reflects concentrations of a protein important in regulating the transport of transferrin iron into cells (Baynes, 1996). Raised sTfR concentration is a useful early indicator of iron deficiency erythropoiesis or tissue (early functional) iron deficiency (Suominen et al., 1998; Beguin, 2003). In a state of iron deficiency, sTfR becomes quickly elevated, whereas it remains
normal in an iron-replete state (Cook et al., 1993; Kamer et al., 2012). STfR is not affected by age or sex (Flowers et al., 1989) and is very useful as it is not affected by the acute-phase response (World Health Organization, 2001). It is also a useful indicator of non-iron deficiency related anaemia, as concentrations do not increase in individuals with other types of anaemia (Beguin, 2003). STfR is best used along with ferritin as a ratio to determine the level of tissue iron deficiency (body iron) (Cook et al., 2003).

**Body iron** is estimated using the ratio of STfR and ferritin described by the formula (Cook et al., 2003):

\[
\text{Body iron (mg/kg)} = \frac{10^{\log_{10}\left(\frac{\text{STfR} \times 1000}{\text{ferritin}}\right) - 2.8229}}{0.1207}
\]

This ratio provides a useful measure of tissue iron in determining the stage between iron depletion (assessed via ferritin) and iron deficiency anaemia (assessed via haemoglobin) (Cook et al., 2003). A positive body iron value suggests tissue iron sufficiency, whereas a negative body iron value suggests a deficit in tissue iron (Cook et al., 2003). A limitation of body iron is that that it has only been validated in adults (Cook et al., 2003), and not in infants. Regardless of this, it has been used to determine the prevalence of iron deficiency in very young children in the United States (US) and is now used in the NHANES survey (Cogswell et al., 2009).

Other indices that are useful in determining iron status include: plasma iron, transferrin saturation, zinc protoporphyrin, and mean cell volume. **Plasma iron** has several limitations (Domellöf et al., 2014). Plasma iron is affected by diurnal variation, where values are higher in the morning and lowest in the evening, therefore the best use of this measure is in the morning after overnight fasting (Archer et al., 2015). It is also affected by recent food or supplement intake, and inflammation and infection (World Health Organization, 2004a).

**Transferrin saturation** is the ratio of plasma iron to total iron binding capacity, and is a more useful marker of iron deficiency than plasma iron alone, as it becomes depleted in the early stages of iron deficiency and is a more stable index (Domellöf et al., 2014). However, both plasma iron and transferrin saturation display overlap between normal iron status and iron deficiency, and
therefore are not sensitive indices in determining iron deficiency (World Health Organization, 2001).

**Zinc protoporphyrin**, also known as erythrocyte protoporphyrin, can be used to detect functional iron deficiency (World Health Organization, 2004a). In the final stage of the synthesis of a haem molecule, iron must bind with protoporphyrin (Fairweather-Tait, 2004). When there is a shortage of iron for this binding, zinc is used as an alternative substrate to bind with protoporphyrin instead of iron (World Health Organization, 2004a), therefore resulting in an increase in zinc protoporphyrin in the absence of sufficient iron (Fairweather-Tait, 2004). However, high values can also be indicative of lead poisoning rather than iron deficiency (World Health Organization, 2004a).

**Mean cell volume** is a measure of red blood cell volume and provides an indication as to whether microcytic or macrocytic anaemia is present, which is useful in differentiating between iron deficiency (microcytic) and other nutrient deficiencies such as Vitamin B12 or folate (macrocytic) (World Health Organization, 2004a). However, this measure is not entirely specific to iron deficiency as it is also affected by thalassaemia (World Health Organization, 2004a).

In summary, multiple indices are needed for the interpretation of iron status (see Section 2.5.2). It is important to note that the determination of iron status is complicated by the presence of infection and inflammation (see Section 2.5.3), the difficulty in differentiating between iron deficiency anaemia and other anaemia, and the fact that the cut-offs for defining iron status in very young children are not well defined (European Food Safety Authority, 2013). It can also be challenging to collect blood samples from very young children because their higher percentage of body fat makes it difficult to find their veins, they often dislike sitting still, and parents may also be reluctant to allow blood sampling in very young children.
2.5.3 Inflammation and infection

The most commonly used acute-phase proteins for determining the presence of inflammation or infection are: C-reactive protein (CRP), and α1-acid glycoprotein (AGP). CRP responds quickly (acute) to infection and inflammation but also drops quickly, whereas AGP responds slowly (chronic) but remains higher for longer than CRP (World Health Organization, 2004a). Thus it is usually recommended that both indices are measured (World Health Organization, 2004a; Suchdev et al., 2016). Although it is less common for very young children in high-income countries to be exposed to infections such as parasites (malaria), a high likelihood of inflammation caused by respiratory or other infections remains (Cross et al., 2009). There appears to be no consensus on the choice of method for removing the effect of infection or inflammation on iron status (Suchdev et al., 2016). Thurnham et al. (2010) suggests adjusting ferritin concentrations if CRP and/or AGP are elevated (i.e. CRP >5 mg/L and/or AGP >1 g/L). However, other methods have been proposed such as: excluding individuals with inflammation, using a higher cut off for ferritin in a population with high rates of inflammation, stratification by level of inflammation, or using statistical methods such as regression modelling for adjustment (Suchdev et al., 2016).

2.5.4 Definitions of stages of iron deficiency

Three stages of iron deficiency are used to describe the iron status of a population: iron depletion, iron deficiency without anaemia (also known as early functional iron deficiency, iron deficiency erythropoiesis, or functional iron deficiency), and iron deficiency anaemia (Gibson, 2005c). The depletion of iron through the stages is caused by either an inadequate intake (or absorption) of iron, or excessive iron losses (Gibson, 2005c). It becomes more difficult to determine the stage of iron deficiency in individuals with a coexisting condition such as chronic disease or inflammation, as the existence of such conditions can alter iron metabolism, leading to changes in iron balance which are not related to insufficient intake or losses of iron (Archer et al., 2015). The stages of iron
Deficiency are often defined using multiple indices (multi-parameter model) where at least two of three indices appear abnormal (as an example see Figure 2.1) (Suominen et al., 1998; Gibson, 2005c). The WHO specifically recommend that haemoglobin, ferritin, and sTfR should be used when determining the stages of iron deficiency (World Health Organization, 2004a). The body iron model (Section 2.5.2) combines sTfR and plasma ferritin and has been shown to be less influenced by inflammation than the ferritin model (a multi-parameter model) (Cogswell et al., 2009). This model has recently been used in children (Cogswell et al., 2009), and adults (Richardson et al., 2015).

![Iron Deficiency Stages Diagram](image)

**Figure 2.1** The effect on common biochemical measures of iron status through the stages of iron deficiency, adapted from Suominen et al. (1998)

**Stage 1: Iron depletion**

During the first stage of iron depletion, iron stores (measured by plasma ferritin) are below the reference range for a given age, because the supply of iron to the body has fallen below the body's requirements (World Health Organization, 2014). During this stage of depleted iron stores, no other
biochemical markers for iron status are significantly affected (World Health Organization, 2014).

**Stage 2: Early functional iron deficiency (without anaemia)**

In early functional iron deficiency, elevated concentrations of sTfR occur in the presence of low ferritin as iron stores are no longer sufficient to meet the need of body tissues for iron (Skikne et al., 1990). However, anaemia does not occur as haemoglobin remains within the normal reference range, although it may have begun to decrease slowly (Gibson, 2005c).

**Stage 3: Iron deficiency anaemia**

Iron deficiency anaemia occurs when erythrocyte production is reduced, and haemoglobin concentrations are reduced below the cut off for a particular age and sex (World Health Organization, 2001). Although anaemia occurs when haemoglobin falls below the recommended reference range for any age, low haemoglobin alone is not a good measure of iron deficiency related anaemia, as other nutritional deficiencies and diseases can lead to low haemoglobin concentrations (World Health Organization, 2014). Multiple biochemical criteria are often used to determine iron deficiency anaemia, such as the use of a multi-parameter model (Figure 2.1). However, as different indices and cut-offs are used by different agencies and researchers, comparisons between studies are challenging.

Studies have consistently demonstrated a pyramid type distribution of iron status such that iron depletion is more common than early functional iron deficiency which in turn is more common than iron deficiency anaemia (Looker et al., 1997; Karr et al., 1996; Soh et al., 2004). However, often only two stages are reported: iron deficiency (effectively iron depletion) and iron deficiency anaemia, due to difficulties assessing biomarkers for determining functional (tissue) iron deficiency (Eussen et al., 2015) (e.g., lack of agreement on which indices are appropriate, difficulties in accessing indices that are not used clinically). In light of the WHO recommendation to use haemoglobin, ferritin,
and sTfR for assessing the iron status of a population (World Health Organization, 2004a), the body iron model may provide a better measure of the continuum of iron status in a population (Cogswell et al., 2009). The challenge will be to make a standard assay for sTfR more widely accepted, available, and affordable.

2.5.5 Iron status of New Zealand toddlers

The prevalence of iron deficiency and iron deficiency anaemia have been reported to be 4-20% among healthy NZ infants and toddlers (6-24 months of age) (Wham, 1996; Soh et al., 2004; Grant et al., 2007) (Table 2.5). However, it is important to note that these studies are limited by their use of different cut offs and indices, and in many cases not accounting for inflammation or infection. Direct comparison of serum ferritin concentrations across studies is also difficult because of variation in how the data are reported – either as medians (Wham, 1996; Grant et al., 2007), means (Soh et al., 2004), or geometric means (Szymlek-Gay et al., 2009) – but appear to be between 16 and 23 μg/L.

Studies have demonstrated that the risk of iron deficiency differs within the NZ population, depending on ethnicity (Soh et al., 2004; Grant et al., 2007). Grant et al. (2007) reported that infants of Māori, Pacific and other non-European ethnicities were at higher risk of iron deficiency in univariate associations, however these associations did not remain in the multivariate model (Grant et al., 2007). Another observational study in NZ infants and toddlers found a positive association between Caucasian ethnicity and serum ferritin concentrations when compared with non-Caucasian ethnicity (Soh et al., 2004). A potential reason for these associations could be a reflection of cultural differences in the iron intakes of these very young children, and also differences in their growth rates.
2.6 Zinc intakes in very young children

2.6.1 Food sources of zinc and its absorption modifiers

The amount of zinc transferred through breast milk is small and decreases from birth into toddlerhood (Brown et al., 2004). Therefore, the intake of complementary foods high in zinc is important in ensuring adequate zinc intakes (Brown et al., 2004). The highest food sources of zinc are: offal (liver, kidney: ~3.0-6.4 mg/100 g), red meat (beef, lamb: ~4.2-5.8 mg/100 g), other meat (chicken, pork: ~1.1-2.4 mg/100 g), seafood (fish etc.: ~0.6-1.9 mg/100 g), eggs (~1.1 mg/100 g), and dairy (milk, cheese: ~0.3-3.2 mg/100 g) (Ministry of Health, 2010).

Increasing amounts of zinc in a meal (including: human milk, formula, and cow’s milk) is known to reduce the fraction of zinc that is absorbed, with a saturation level of intake of around 4.6-5.2 mg of zinc per meal, so that the total zinc absorption from a meal is on average around 25% (Sandström, 1992). Although higher protein intakes are known to increase zinc absorption by counteracting the inhibitory effect of phytate, this is thought to be dependent on the type of protein ingested (Lönnerdal, 2000). It was earlier suggested that casein in milk may negatively affect zinc absorption (Sandström et al., 1983), however, a small study in adults did not find any significant effect of casein intake on zinc absorption (Davidsson et al., 1996). Also, a small (six male infants) cross-over study assessed two formulas (casein vs. cow’s milk) with equivalent zinc concentrations, and the authors reported significantly higher total absorption (calculated as fractional absorption, multiplied by the total daily dietary zinc) of zinc in the casein based formula compared with the cow’s milk based formula ($p=0.02$) (Krebs et al., 2000). However, as infants were not fed complementary foods in this study (Krebs, 2000), it is not known whether the introduction of complementary foods would impact this finding.

The strongest dietary factor known to inhibit the absorption of zinc is phytate (Brown et al., 2004). While high calcium intakes are known to impair
zinc absorption, it has been suggested that this only occurs in the presence of high phytate intakes (Gibson et al., 2010). The use of the phytate-to-zinc molar ratio is thought to provide a better measure of zinc bioavailability than a ratio including calcium (Lönnerdal, 2000). Foods high in phytate are cereals (e.g., wheat, rice, maize), nuts, seeds, and legumes, and high intakes of these foods, are thought to inhibit the absorption of zinc (Lönnerdal, 2000). Due to commercial processing, the phytate content of pre-prepared infant foods is much lower, improving overall mineral absorption in high-income countries (Gibson et al., 2010). It is important to note that there is some controversy over whether dietary phytate inhibits zinc absorption in infants and children as it does in adults (Miller et al., 2015), although no studies have yet specifically investigated the impact of different concentrations of phytate on zinc absorption in healthy infants.

The International Zinc Nutrition Consultative Group (IZiNCG) recommends the use of the phytate-to-zinc molar ratio as the best way of assessing and predicting zinc bioavailability from the diet (Brown et al., 2004). This is calculated as:

\[
\text{Phytate-to-zinc molar ratio: } \frac{\text{phytate (mg)}}{660} / \frac{\text{zinc (mg)}}{65.4}
\]

The WHO (World Health Organization, 1996) proposed absorption estimates based on studies assessing zinc absorption from either single meals or the total diet, although, it is not known if these absorption estimates are applicable to very young children (Gibson et al., 2010):

- High availability (50% absorption) = phytate-to-zinc molar ratio <5
- Moderate availability (30% absorption) = phytate-to-zinc molar ratio 5-15
- Low availability (15% absorption) = phytate-to-zinc molar ratio >15

It appears that phytate content impacts the most on zinc absorption, and it is recommended to use the phytate-to-zinc molar ratio when estimating bioavailable zinc from the diet (Brown et al., 2004). However, increasing amounts of zinc in a meal is also known to reduce the amount of zinc that is
absorbed (Sandström, 1992). A summary of absorption modifiers and potential absorption modifiers of zinc is presented in **Table 2.6**.

**Table 2.6** Summary of dietary factors shown to modify zinc absorption, and dietary factors that may modify zinc absorption

<table>
<thead>
<tr>
<th>Intake modifiers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enhances zinc absorption</strong></td>
<td></td>
</tr>
<tr>
<td>Increased animal protein</td>
<td>Lönnerdal (2000)</td>
</tr>
<tr>
<td><strong>Inhibits zinc absorption</strong></td>
<td></td>
</tr>
<tr>
<td>Increased zinc (total)—does not inhibit but a</td>
<td>Sandström (1992)</td>
</tr>
<tr>
<td>saturation of absorption occurs</td>
<td></td>
</tr>
<tr>
<td>Iron (as a supplement only)</td>
<td>Lönnerdal (2000)</td>
</tr>
<tr>
<td>Calcium (in presence of high phytate intakes)</td>
<td>Ferguson et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>Lönnerdal (2000)</td>
</tr>
<tr>
<td></td>
<td>Gibson et al. (2010)</td>
</tr>
<tr>
<td>Phytate (however, there are inconsistencies regarding</td>
<td>Brown et al. (2004)</td>
</tr>
<tr>
<td>the impact on zinc absorption in infants)</td>
<td>Miller et al. (2015)</td>
</tr>
<tr>
<td><strong>Inconclusive potential inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>Sandström et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Davidsson et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Krebs et al. (2000)</td>
</tr>
</tbody>
</table>

### 2.6.2 Zinc intakes of very young children in New Zealand

Assessing the dietary intake of zinc as well as absorption modifiers in populations is important when assessing the risk of zinc deficiency, as inadequate zinc intake is thought to be one of the most likely causes of deficiency (Brown et al., 2004). Despite a large number of studies assessing the zinc intakes of very young children internationally, few studies have been conducted in NZ infants and toddlers (Heath et al., 2002; Ferguson et al., 2004; Morgan et al., 2010; Morison et al., 2016).

A longitudinal study by Heath et al. (2002) reported median (25th and 75th percentiles) zinc intakes in 9 month old infants to be 4.0 (3.5, 5.1) mg per day and 4.5 (3.9, 5.3) mg per day in 12 month old toddlers. At 18 and 24 months, median intakes were 5.2 and 5.0 mg per day, respectively. Limitations of this study included: using an estimation of breast milk intakes (674 mL per day for
infants, and 467 mL per day for toddlers), and the use of 24-hour recall (at 9 months) and three-day diet records (all other time points) which could produce inconsistencies in the estimation of actual intakes.

Ferguson et al. (2004) assessed a large number (n=323) of non-breastfeeding infants (6-11 months) and toddlers (>12 months) and found the mean (SD) zinc intakes were 4.8 (1.5) mg per day. The main sources of zinc in their diets were infant formula/dairy products (36%), and cereals (15%).

A randomised controlled trial by Morgan et al. (2010) assessed the zinc intakes of 225 toddlers (12-20 months) at baseline by intervention group (meat group, fortified toddler milk group, or non-fortified toddler milk group). Overall mean (SD) intakes of the three groups were 4.6 (1.2), 5.0 (0.8), and 5.2 (1.3) mg per day, with very low prevalences of inadequate intakes of 0.4%, 0%, and 3.9%. At the end of the intervention, zinc intakes increased significantly in the meat and fortified toddler milk groups, compared with the Control group. However, due to the high proportion of Caucasian ethnicity (80%), these intakes may not be reflective of the wider NZ population, particularly those of other ethnicities where there may be cultural differences in the dietary intakes of toddlers.

More recently, an observational study assessing the differences in intake between NZ infants (6-8 months) following traditional spoon-feeding and BLW reported the geometric mean (95% CI) zinc intakes of spoon-fed infants to be 3.7 (3.3, 4.1) mg per day, however, the intakes in BLW infants were significantly lower with a geometric mean (95% CI) intake of 3.0 (2.6, 3.3) mg per day (p=0.001). The authors did not assess the zinc intakes of toddlers (>12 months of age) in this study (Morison et al., 2016).

Overall, these studies agree that the zinc intakes of very young children (aged 6-24) in NZ are between 3.0 and 5.2 mg per day (Heath et al., 2002; Ferguson et al., 2004; Morgan et al., 2010; Morison et al., 2016). One of these studies reported a low prevalence (0-4%) of zinc intakes (Morgan et al., 2010), suggesting that zinc intakes in very young children are adequate.

Table 2.7 describes the studies reported in Sections 2.6.2 and 2.7.3 and provides more detail including the findings regarding the zinc intakes and status of very young children in NZ, and the limitations of each study.
Table 2.7 Studies assessing the zinc intakes and/or status of very young children in New Zealand (from year 2000 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
</table>
| Heath et al. (2002) | n = 74        | Longitudinal study. Demographic information at initial visit. Weekly telephone calls to determine information on infant feeding. **Intake**: Estimated 24h diet record (prior to each monthly visit). 3DDR at 12, 18 and 24 months. **Status**: Not reported. | **Zinc intake**
Median (25th, 75th percentiles), mg/day:
9 months: 4.0 (3.3, 5.1).
12 months: 4.5 (3.9, 5.3).
**Zinc status**
Not reported. | • Did not calculate BM intake - estimated BM intake based on previous published work.
• Socioeconomically advantaged group compared to the general population of NZ.
• Use of 24h diet recall may have resulted in memory lapses of what was actually consumed. For some time points only 24h recall data was used which is not a good reflection of ‘usual’ intake.
• Used estimated rather than weighed diet records which may lead to under- or over-estimation of intake.
• No infant biochemical zinc status data. |
Table 2.7 (continued) Studies assessing the zinc intakes and/or status of very young children in New Zealand (from year 2000 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Main findings</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferguson et al.</td>
<td>n = 323</td>
<td>Cross-sectional. Anthropometric and socioeconomic status information collected.</td>
<td>Zinc intake Mean (SD), non-BF children (n=230): 4.8 (1.5) mg/day. Zinc status Mean (95% CI) (n=172): 10.6 (9.6, 11.9) μmol/L. Prevalence of low zinc status = 11%.</td>
<td>• This is a published abstract from a conference. Results were not published elsewhere, therefore, there is limited methodological data available for this study – not known at what exact age blood sample was taken or when intake was assessed. • Not known if trace element free techniques were used.</td>
</tr>
<tr>
<td>Morgan et al.</td>
<td>n = 225 (all data) Very young children 12-20 months. Recruited through newspaper advertisements, flyers, letters sent to families through newspaper birth notices.</td>
<td>Randomised placebo controlled trial. Randomised into meat group n=90, fortified toddler milk group n=45, or non-fortified milk placebo group n=90. Demographic questionnaire. Weight and length. Intake: 3-day WDR at baseline, week 4 and week 18 of the intervention. Status: Non-fasting venous blood samples at baseline and post intervention. -Used trace-element free techniques.</td>
<td>Zinc intake Mean (SD) at baseline, mg/day: Placebo group: 5.2 (1.3). Meat group: 5.0 (0.8). Fortified milk group: 4.6 (1.2). Zinc status Geometric mean (95% CI) at baseline, μmol/L: Placebo group: 9.8 (9.5, 10.2). Meat group: 9.5 (9.2, 9.8). Fortified milk group: 10.0 (9.4, 10.5). Prevalence of low zinc status at baseline = 30% placebo, 48% meat group, 43% fortified milk group.</td>
<td>• Study sample may not be representative of the multi ethnic population of NZ – as a high percentage of Caucasian infants were included (80%).</td>
</tr>
<tr>
<td>Authors</td>
<td>Sample Size</td>
<td>Study Design</td>
<td>Intake</td>
<td>Zinc Intake</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------</td>
<td>--------------</td>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>Morison et al. (2016)</td>
<td>n = 51</td>
<td>Cross-sectional</td>
<td>Questionnaires and either a 3-day or 1-day WDR. BLW definition = based on question asked after completing WDR “what approach to infant feeding were you using’ – ‘spoon-feeding’ or ‘BLW’.</td>
<td>Geometric mean (95% CI), mg/day: Full BLW: 3.0 (2.6, 3.3). TSF: 3.7 (3.3, 4.1). Zinc intakes were significantly lower in BLW compared with TSF group. Zinc status Not reported.</td>
</tr>
</tbody>
</table>

- Small sample size.
- Potential recruitment bias.
- No infant biochemical zinc status data.

Abbreviations: 3DDR, three-day diet record; BF, breastfed; BLW, Baby-Led Weaning; BM, breast milk; h, hour; TSF, traditional spoon-feeding; WDR, weighed diet record.
2.7 Zinc status of toddlers

2.7.1 Why is the zinc status of toddlers important?
During childhood, the risk of zinc deficiency is increased (Gibson et al., 2011). This occurs because there is a higher physiological requirement for zinc due to the high growth rate during infancy (Brown et al., 2004), but typical complementary foods (e.g., fruit and vegetables) are also generally low in zinc (World Health Organization, 2004b). Furthermore, after 6 months of age breast milk no longer provides sufficient amounts of zinc (World Health Organization, 2004b). Suboptimal zinc status during childhood may increase the risk of infection (Shankar et al., 1998; Fraker et al., 2000), and have detrimental effects on growth (Nissensohn et al., 2016).

2.7.2 Indices for determining zinc status

Plasma/serum zinc

Plasma/serum zinc concentration is the most widely used marker for assessing the biochemical zinc status of populations (Brown et al., 2004; De Benoist et al., 2007) and is thought to reflect usual dietary zinc intakes from the previous few weeks to months (IZiNCG, 2007). Plasma/serum zinc concentration reflects dietary intake, responds to zinc supplementation, and has interpretive data, which therefore make it a useful marker for assessing the zinc status of a population (IZiNCG, 2007). The term ‘plasma’ zinc will be used to refer to plasma or serum in this literature review.

IZiNCG have set interpretive criteria for determining the risk of zinc deficiency in a population using plasma zinc (IZiNCG, 2007). The criteria were based on data from the US NHANES II survey of healthy males and females aged 3-74 years, where reference curves were established for the 2.5th percentile for specific age groups and accounted for factors affecting plasma zinc concentrations (age, sex, fasting status, and time of day) (Hotz et al., 2003). For
all infants and children under the age of 10 years the suggested cut offs for defining low plasma zinc concentration are 9.9 μmol/L for blood samples collected in the morning and 8.7 μmol/L for those collected in the afternoon (Hotz et al., 2003).

The use of plasma zinc does however have limitations such as: the available age groups for interpretive data, and methodological and collection factors. Because the cut offs were established using data from healthy individuals from 3 years of age, we do not know whether a different cut off is needed for infants (0-12 months) and toddlers (12-24 months) who fall below 3 years. Also, methodological factors (such as: recent food intake, infection and inflammation, and adventitious contamination) affect the accuracy of plasma zinc measurements (Gibson et al., 2008) and therefore it is important that blood samples are collected and analysed using a standardised protocol in order to eliminate, or at least minimise, any confounding factors. In particular, it is important to rule out any influence of inflammation (Brown et al., 2004). This can be done by measuring an acute phase protein (often CRP and/or AGP), which can be used as controlling variable in the statistical analyses (King, 2011; Raiten et al., 2015). Ideally, inflammation would be excluded prior to analysis by collecting blood samples only from healthy individuals. Finally, it can be challenging to collect blood samples from very young children because their higher percentage of body fat makes it difficult to find their veins, and they often dislike sitting still.

Other indices

A number of other indices have been proposed, but are not widely used for assessing zinc status in populations. They also have their benefits and limitations.

Hair zinc has been proposed as a useful measure of long-term zinc status (Brown et al., 2004). Zinc concentrations in hair samples are less affected by recent food intake, diurnal variation, and infection and inflammation than venipuncture and collection of hair samples is also less invasive (Brown et al., 2004). However, hair zinc samples are affected by age, sex, season, hair growth
rate, and adventitious contamination (Gibson et al., 2008; King et al., 2016), and limited reference data are available for comparisons (Brown et al., 2004).

Other measures which may be useful include: urinary zinc, nail zinc, zinc-dependent proteins, white blood cell zinc, oxidative stress and DNA integrity biomarkers, zinc kinetics, and functional biomarkers such as taste acuity and neurobehavioural function (Gibson et al., 2008; Raiten et al., 2015). However, very little research has determined the feasibility and validity of these potential biomarkers (Gibson et al., 2008).

To conclude, there is a need for a more specific and sensitive biomarker for assessing population zinc status. Currently, plasma zinc is the method most widely used to assess the biochemical zinc status of populations. Although the reference data available are based on data from 3-10 year olds so may not be appropriate to provide a conclusion regarding zinc status for the age group presented in this thesis, they are the only values currently available.

2.7.3 Biochemical zinc status of New Zealand toddlers

Although very early childhood is a vulnerable time when there is a high risk of zinc deficiency (Gibson et al., 2011), there is a scarcity of studies reporting biochemical zinc status for this age group in NZ.

A study in NZ toddlers (n=225) aged 12-20 months reported a geometric mean (95% CI) serum zinc concentration of 9.8 (9.2, 10.5) μmol/L, after excluding participants with infection and adjusting for time of day of blood collection (Morgan et al., 2010). There was a high mean percentage (30-48%) of biochemical zinc deficiency, which would suggest that mild zinc deficiency was prevalent. However, dietary zinc intakes were adequate and the growth rates of the infants were normal, suggesting that the biochemical results may be overestimating zinc deficiency. Ferguson et al. (2004) reported a much lower prevalence of zinc deficiency (11%), however, this study was in a wider age range of infants and toddlers (6-24 months), who had a slightly higher mean (95% CI) serum zinc concentration of 10.6 (9.6, 11.9) μmol/L, compared with the previous study. The study results by Ferguson et al. (2004) were published
in an abstract and it is not known at what exact age blood samples were collected, and therefore what age these zinc status data represent.

The results of these studies suggest that borderline zinc status is common during very early childhood in NZ. However, they also highlight the lack of agreement between biochemical status and dietary zinc intakes, which appear to be adequate (Section 2.6.2). This lack of agreement has led to questions about whether the cut offs used for determining both zinc intakes and zinc status are appropriate for this age group.

### 2.7.4 Factors associated with biochemical zinc status in very young children

Table 2.8 describes the studies reported in this section and provides more detail including specific associations found, and the limitations of each study.

#### Biological and social factors

The relationship between age and biochemical zinc status is controversial. Studies in infants (Brown et al., 1993; Michaelsen et al., 1994; Hemalatha et al., 1997) and children (Thurlow et al., 2005; Gibson et al., 2010; Galetti et al., 2016) have found an association between age and zinc status. However, other studies including those in very young children (Karr et al., 1997; Bouglé et al., 2000), and preschool, and school age children (Gibson et al., 2000; Kongsbak et al., 2006) have found no association between age and zinc status. The differences may be due to the differences in the age ranges assessed in these studies.

An association between zinc status and sex has been consistently reported in school-age children from both low income and high income countries such that more males than females have lower serum zinc concentrations (Cavan et al., 1993; Thurlow et al., 2005; Qin et al., 2009; Gibson et al., 2010; Fiorentino et al., 2013; Houghton et al., 2016). However, the majority of studies in infants and toddlers have found no association between biochemical zinc status and sex (Brown et al., 1993; Michaelsen et al., 1994; Karr et al., 1997; Persson et al., 1998). One study in infants reported similar
findings to those in children in that males had lower zinc status than females but this was only at 2 months of age and the proportion of males versus females included was not reported (Salmenperä et al., 1994). It is considered that males tend to have lower zinc status due to a higher zinc requirement because of their higher percentage of lean body mass, as well as their faster growth rate, factors which become more relevant as children go through puberty (Brown et al., 2004). The association seen between sex and plasma zinc in children may reflect differences in growth rates during childhood due to growth spurts occurring at different ages, in contrast to infancy when males and females tend to grow at relatively similar rates (Hotz et al., 2003).

Whether there are associations between biochemical zinc status and growth (weight and length) is controversial for this age group. Some studies in very young children have found no association between biochemical zinc status and measures of growth (Salmenperä et al., 1994; Persson et al., 1998; Bouglé et al., 2000), although limitations of these studies include small sample sizes and failure to follow IZiNCG protocols. The authors of one study found a negative association between biochemical zinc status at 2 months and weight and knee-heel length in the following month in healthy infants. However, at 9 months, a positive association was seen between the same measures and biochemical zinc status (Michaelsen et al., 1994). Other studies reported zinc status to be negatively associated with weight-for-age z-score (Ferguson et al., 2004) and positively associated with length-for-age z-score (Brown et al., 1993) in very young children aged between 6-24 months. There is a convincing mechanism for associations between zinc status and growth during infancy - the demand for zinc for the synthesis of tissue during periods of rapid growth could deplete the serum zinc pool (Michaelsen, 1997).

Very few studies have assessed whether ethnicity is associated with biochemical zinc status. One study in NZ school children reported that those of Pacific ethnicity had a higher prevalence of low serum zinc concentrations than children of Māori or New Zealand European and Other (NZEO) ethnicities (Gibson et al., 2010).
Studies appear to agree that there is an association between Socio-economic status (SES) and plasma zinc concentrations in infants, toddlers, and children, in that those with low SES are more likely to have low plasma zinc concentrations (Villalpando et al., 2003; Thurlow et al., 2005; Arsenault et al., 2011; Engle-Stone et al., 2013; Galetti et al., 2016). This is despite different methods being used to describe SES. One study found no association between SES and biochemical zinc status in NZEO children, but this may be due to the large amount of missing data for SES (n=77), reducing power to determine any association (Gibson et al., 2010).

**Biochemical factors**

It is generally agreed that there is a relationship between inflammation or infection (measured by acute phase proteins) and zinc status in healthy infants and children. Elevated CRP concentrations have been associated with lower plasma zinc concentrations in infants (Brown et al., 1993; Arsenault et al., 2011) and children (Thurlow et al., 2005; Kongsbak et al., 2006; Engle-Stone et al., 2014; Galetti et al., 2016), although some studies in school children have found no association between CRP and serum zinc concentrations (Qin et al., 2009; Gibson et al., 2010; Bui et al., 2012).

There is currently no agreed approach for accounting for inflammation and infection when assessing zinc status in populations, although several approaches have been proposed (Raiten et al., 2015). IZiNCG recommends statistical adjustment or exclusion (although exclusion may lead to selection bias) (IZiNCG, 2007). Failure to account for the impact of inflammation or infection could lead to an overestimation of zinc deficiency in a population with a high prevalence of infection (Tomkins, 2003; Suchdev et al., 2016).

There is a scarcity of research investigating whether there is an association between serum selenium concentration and serum zinc concentration. This is of interest because selenium is important for immune function and may compromise zinc status (Maret, 2000; Lyons et al., 2004), and selenium status tends to be low in some countries, including NZ (Thomson, 2004). An observational study in children (5-15 years) found that serum
selenium concentrations predicted serum zinc concentrations in those self-reporting as NZEO or Pacific ethnicity but not those of Māori ethnicity (Gibson et al., 2010). This may be due to cultural differences in dietary intakes.

Krittaphol et al. (2006) reported a strong correlation between serum selenium and serum zinc concentrations ($r=0.216$, $p<0.001$) in Thai children (aged 6-13 years). To the Candidate’s knowledge, there are no studies assessing this association in very young children.

Previous studies have reported that infants and children with anaemia (categorised as low haemoglobin) had significantly lower serum zinc concentrations than those without anaemia (Ece et al., 1997; Gürgöze et al., 2006; Angelova et al., 2014; Houghton et al., 2016). As iron and zinc are found in similar foods it could be that intakes will decrease simultaneously and therefore deficiency may also occur simultaneously. However, the authors of one study found no association between serum zinc and ferritin and therefore concluded that the association between zinc and haemoglobin is not due to the food supply, as would be expected (Houghton et al., 2016). Therefore, the mechanism behind the association between anaemia and low biochemical zinc is not yet known.

**Methodological factors**

Some methodological factors (particularly time of the day and fasting status) are known to be associated with serum zinc concentrations. A number of studies have reported that serum zinc concentrations are higher when biochemical samples are collected in the morning compared with the afternoon (Gibson et al., 2010; Arsenault et al., 2011; Engle-Stone et al., 2014; Galetti et al., 2016), and are also affected by fasting status (Arsenault et al., 2011; Engle-Stone et al., 2014; Galetti et al., 2016). Because of this, it has been recommended that these factors are controlled for when collecting blood samples to determine plasma zinc concentrations in populations (IZiNCG, 2007).

Other less conclusive methodological factors that may be associated with biochemical zinc status are season and use of topical zinc lotions. Season has been shown to be associated with hair zinc concentrations (Gibson et al., 1989; Wilhelm et al., 1991), although this could be reflective of different zinc intakes.
in different seasons. Very little research has looked at whether there is also an association with biochemical zinc status. One study reported higher serum zinc concentrations during the winter compared with summer, although this was only shown to be significant in a subgroup of children of Pacific ethnicity (Gibson et al., 2010). Zinc oxide is the most common active ingredient found in zinc containing topical lotions used to treat and prevent nappy rash in infants, and it is possible that it may be absorbed through the skin. However, very few studies have assessed whether there is a relationship between zinc status and use of zinc containing topical lotions. One study by Derry et al. (1983) reported an increase in serum zinc concentrations one hour after application of zinc oxide (40%) containing lotion. However, serum zinc concentrations remained constant over a 10-day period, which suggests that the use of lotions containing zinc oxide (40%) is unlikely to increase zinc status. This study was limited by its small sample size (n=6), and by only including adult males. No studies have assessed an association in healthy infants and toddlers.

**Dietary factors**

There are few studies that have reported a relationship between dietary zinc intake and biochemical zinc status during infancy. However, this may be impacted by the difficulty of collecting accurate dietary data in very young children. Many studies have reported no association between dietary zinc intake and biochemical zinc status (Michaelsen et al., 1994; Kattelmann et al., 2001; Taylor et al., 2004; Han et al., 2011; Cantoral et al., 2015). Conversely, Salmenperä et al. (1994) found that biochemical zinc status was positively associated with daily zinc intakes at 6, 9 and 10 months, although zinc intakes were only calculated from breast milk and not complementary foods. Angelova et al. (2014) also found a significant positive correlation between plasma zinc and zinc intakes.

Few studies have assessed meat intake as an individual food group in very young children (Taylor et al., 2004; Morgan et al., 2010). These studies reported no association with biochemical zinc intake, at least in toddlers (Taylor et al., 2004; Morgan et al., 2010), even though red meat is a rich source
of highly bioavailable zinc (Jalla et al., 2002). This may be because red meat intakes are either low in this age group, as shown in a study of 18 month old Australian infants where intake of red meat was on average less than 10 g per serve (Webb et al., 2005), or that there is methodological difficulty in the assessment of meat intakes in this age group as often meat is consumed as part of ‘mixed dishes’ rather than alone, or both. An association between flesh foods intake and hair zinc concentrations has been seen in Canadian children aged 4-5 years (Vanderkooy et al., 1987). While it might be expected that the intake of **cow’s milk** and **dairy** products would be associated with zinc concentrations, as they are good sources of zinc, at least in comparison to fruits and vegetables (Ministry of Health, 2010). To the Candidate’s knowledge, this has not been directly examined in relation to biochemical zinc concentrations in very young children.

As **phytate** is a powerful inhibitor of zinc absorption, at least in adults (Brown et al., 2004), it would be expected that intakes of phytate or the phytate-to-zinc molar ratio would be associated with biochemical zinc concentrations, particularly if the intake of phytate is high. Only one study, in Guatemalan children, has found an association between high phytate intake and low hair zinc concentration (Cavan et al., 1993). However, this has not been assessed in very young children, or with biochemical zinc concentrations.

Only one study has assessed the relationships between the timing of complementary food introduction and zinc status (Kattelmann et al., 2001). No association was found between early or late **introduction to complementary foods** and serum zinc status in formula fed infants at 12 months of age (Kattelmann et al., 2001). As this study was limited to assessing formula fed infants, results may differ in breastfed infants due to the low concentrations of zinc in breastmilk (Brown et al., 2004), however this has not been determined.

Very few studies have reported whether **infant milk type, duration of exclusive breastfeeding** and **total duration of breastfeeding** are associated with zinc status. Lönnerdal et al. (1994) reported no significant differences in the serum zinc concentrations of infants fed either breast milk or zinc supplemented (4 mg/L) infant formulas after either 6 weeks or 6 months of
consuming the milk. This is surprising because breast milk is lower in zinc (~1.2-4 mg/L (Krebs et al., 1995)) than infant formula which is fortified (~2.9-5.8 mg per 100 g zinc) (Ministry of Health, 2010), but may reflect adaptation so that zinc absorption and retention are higher in response to lower intakes (Brown et al., 2004). However, a limitation of the study by Lönnnerdal et al. (1994) is that the sample size of the breast fed group was small (n=10) compared with the formula fed group (n=50), which may have reduced the power to detect a significant association between infant milk intake in zinc status. Another earlier study found no association between the duration of exclusive breastfeeding, or the duration of total breastfeeding, and zinc status in 9 month old infants (Michaelsen et al., 1994).

**Food fussiness** may be associated with zinc status in very young children. Food fussiness (also known as 'picky' eating) is common in very young children (Wright et al., 2007), and a study in American toddlers found that zinc intakes were significantly lower in those considered as 'picky' eaters compared with non-'picky' eaters (Carruth et al., 2004). Therefore, as food fussiness has been shown to be associated with lower zinc intakes, this may also result in lower biochemical zinc status. However, to the Candidate’s knowledge no study has determined whether there is an association between food fussiness and zinc status in very young children.

In conclusion, there is evidence for an association between biochemical zinc status and socio-economic status, ethnicity, inflammation/infection, and methodological factors (time of day and fasting status) during infancy. However, it is less clear whether biochemical zinc status is associated with age, sex, measures of growth, dietary zinc intake, and food fussiness. It is also unclear whether there is any association between biochemical zinc status and plasma selenium concentration in infants and toddlers, as this has currently only been reported in school children.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Country^2</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status^3</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown et al. (1993)</td>
<td>Peru Upper middle Income country. Low (16%) level of stunting.</td>
<td>n = 153 Very young children 11-19 months. Recruited as part of a cohort prospective study assessing oral rotavirus vaccine.</td>
<td>Cross-sectional. Weight and length. <strong>Intake:</strong> Not reported. <strong>Status:</strong> Morning non-fasting venous blood sample. -Used trace-element free techniques.</td>
<td><strong>Zinc intake</strong> Not reported. <strong>Zinc status</strong> Mean (SD) serum zinc: 7.3 (2.1) μmol/L. Prevalence of low zinc status (&lt;9.2 μmol/L) = 80%.</td>
<td>Significantly lower mean zinc concentrations in younger compared with older infants. No association between serum zinc and sex. Serum zinc was significantly associated with LAZ and marginally with WAZ. Serum zinc concentrations significantly lower in infants with elevated CRP. Significant predictors of serum zinc concentrations were: age and LAZ (after controlling for confounders, age, sex, anthropometric status, SES).</td>
<td>• Cross-sectional nature. • Only measured CRP - this measure does not identify chronic inflammation. • Did not assess dietary zinc intake. • Did not use standard IZINCG cut off for low zinc status.</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Income Level</td>
<td>Sample Description</td>
<td>Study Design</td>
<td>Intake:</td>
<td>Status:</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------</td>
<td>--------------</td>
<td>--------------------</td>
<td>-------------------------------</td>
<td>--------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Salmenperä et al. (1994)</td>
<td>Finland</td>
<td>High</td>
<td>Infants 0-12 months. Recruited through a hospital in Helsinki, Finland.</td>
<td>Randomised controlled trial (supplemented mothers only). Length and weight at 0, 2, 4, 6, 7.5, 9, 10, 11 and 12 months.</td>
<td>72h test-weighing BM intakes at 4, 6, 9, 10, 11 and 12 months.</td>
<td>Umbilical blood sample initially then non-fasting venous blood sample at 2, 4, 6, 7.5, 9, 10, 11 and 12 months.</td>
</tr>
</tbody>
</table>

- At 2 months of age serum zinc was lower in boys. Serum zinc concentrations were associated with daily zinc intakes at 6, 9 and 10 months. Serum zinc concentrations were not associated with birth weight or length.

- Did not state how many boys compared with girls were in the study.

- Zinc intake only assessed by BM intake, not complementary foods.

- Did not use standard IZiNCG cut off for low zinc status.
### Table 2.8 (continued)  
Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
</table>
| Lönnerdal et al. (1994) | Sweden  | High income country. Did not report stunting prevalence. | Randomised double-blind controlled trial. Height, weight at birth, 6 weeks and 6 months. **Intake:** Those consuming IF used one of 5 study formulas from 6 weeks until the end of the study – all contained 4 mg zinc. No solid food was allowed before 6 months. **Status:** Fasted venous blood samples at 6 weeks and 6 months. | Zinc intake Not reported. **Zinc status** Mean serum zinc, μmol/L **:** 16.2-19.0 at 6 weeks (baseline). 13.8-18.8 at 6 months. | Baseline data: Similar serum zinc concentrations across groups at both 6 weeks and 6 months. No significant difference in serum zinc concentrations between infants BF or formula fed. Did not look at any other associations. | • Small sample size (only 10 in each group).  
• Did not assess dietary intake (main outcome was up until 6 months).  
• Did not state whether strict zinc protocol was used for preventing contamination – may cause random error.  
• Did not report inflammation or infection. |
Michaelsen et al. (1994) Denmark High income country. Did not report stunting prevalence. $n = 91$ Infants 2-9 months. Recruited as part of the Copenhagen Cohort study on nutrition and growth. Longitudinal observational study. Weight and knee-heel length. **Intake:** 24hr food record. Test-weighing BM intake. **Status:** Non-fasting venous blood samples. **Zinc intake** Mean: ~4.5 mg/day in non-BF infants at 12 months. **Zinc status** Mean serum zinc, $\mu$mol/L: 10.6 at 6 months. 8.3 at 9 months. Decline in serum zinc status from 6 to 9 months. No effect on age. No association between sex and serum zinc, or between interval between last meal and time of blood collection. Duration of EBF, total duration of BF, or zinc intake was not associated with serum zinc at 9 months. **Negative association:** between serum zinc at 2 months and weight, knee-heel length during the following month. **Positive association:** between serum zinc at 9 months and weight, knee-heel length during the following 3 months. Also, IGF-1 at 9 months but not at 2 or 6 months. Weak trend of a positive association between meat/fish (food group) intake and zinc status at 9 months. **Observational nature.** • Intake data only presented as a graph which is difficult to interpret exactly. • Dietary intake data only presented for non-BF infants. • Did not state whether strict zinc protocol was used for preventing contamination – may cause random error. • Blood samples were collected from $n=71$ (78%) at 2 months, $n=58$ (64%) at 6 months, $n=57$ (63%) at 9 months. Fever present in $n=4$ at the time of sampling.
Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
</table>
| Michaelsen, (1997) (Same study group as Michaelsen et al. (1994)). | Denmark High income country. Did not report stunting prevalence. | n = 146 (all data) n=87 study group, n=59 Control group Infants followed from 0-12 months. Recruited through a hospital in Copenhagen, Denmark. | Cohort study. Demographic questionnaire. Weight, crown-heel length, head circumference, triceps and subscapular skinfolds. Intake: 24h food records monthly (at 2 and 4 months: 48h food record collected, at 9 months: 5-day record collected). Test weighing of human milk intake. Status: Blood samples at 2, 6 and 9 months. | Zinc intake
Median (10th, 90th centiles), mg/day:
4.0 (3.0, 5.5) in non-BF infants at 7 months.
4.5 (2.7, 6.8) in non-BF infants at 12 months. Zinc status
Mean serum zinc, μmol/L (from Fig. 16):
~9 at 2 months.
~10.5 at 6 months.
~8.5 at 9 months. | Negative association:
Weight and linear growth velocity (knee-heel length) at 2 months.
Positive association:
Weight and linear growth velocity (knee-heel length) at 9 months. No association between zinc intake and serum zinc or the intake of foods high or low in zinc bioavailability and serum zinc. | • Same limitations as above.
• Supplement of previous paper, which reported outcomes differently.
• Only included non-BF infants in dietary zinc analysis.
• Did not state the type of biochemical sample taken (venous or capillary) or whether strict zinc protocol for preventing contamination were used – may cause random error. |
<table>
<thead>
<tr>
<th>Hemalatha et al. (1997)</th>
<th>India Lower middle income country. Did not report stunting prevalence.</th>
<th>Cross-sectional.</th>
<th>Zinc intake In full term infants concentrations of zinc in BM decreased from 2.3 mg/L colostrum to 1.5 mg/L at 4-6 months to 1.15 mg/L at 7-12 months.</th>
<th>Cross-sectional nature. Did not specify where infants were recruited. Did not assess dietary intake of zinc other than BM. Did not use strict zinc protocol for preventing contamination — may have caused random error.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n=186$ (all data) $n=118$ full term, $n=68$ pre-term. All pre-term and 92 full term infants were BF. Only 26 full term infants were FF. Randomly selected infants.</td>
<td><strong>Intake:</strong> Breast milk samples collected.</td>
<td><strong>Status:</strong> Venous blood samples.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Zinc status</strong> Mean (SE) serum zinc, μmol/L *: <strong>Full term BF:</strong> 11.8 (1.4) at 3 months. 11.3 (0.8) at 6 months. 12.8 (1.2) at 9 months. 17.5 (1.0) at 12 months. <strong>Full-term FF:</strong> 8.7 (0.8) at 3 months. 10.4 (0.5) at 6 months. 10.0 (1.1) at 9 months.</td>
<td>Decline in zinc status from birth until 6 months. From 6 months zinc concentrations began to rise again up to 12 months. Did not look at any other associations.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)\(^1\)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country(^2)</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status(^3)</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
</table>
| Karr et al. (1997) | Australia High income country. Did not report stunting prevalence. | \(n = 467\) Very young children 9-62 months. Recruited as a random sample. | Cross-sectional. **Intake:** Not reported. **Status:** Venous blood sample. Samples were not collected when infants were unwell. | Zinc intake  
Not reported.  
**Zinc status**  
Mean (95% CI) serum zinc, μmol/L:  
At 9-23 months: 13.8 (13.4-14.3).  
At 24-35 months: 13.6 (13.2-14.1).  
At 36-47 months: 13.8 (13.4-14.2).  
At 48-62 months: 14.2 (13.7-14.6).  
Prevalence of low zinc status (<8 μmol/L) = 0%. | Serum zinc was not associated with age or sex.  
Strong correlations between serum zinc concentrations and prealbumin and weak correlations with Vitamin A, and retinol binding protein. | • Cross-sectional nature.  
• Did not assess dietary intake.  
• Age groups were broadly grouped.  
• Did not state whether strict zinc protocol was used for preventing contamination – may cause random error.  
• Did not use standard IZiNCG cut off for low zinc status. |
| Persson et al. (1998) | Sweden High income country. Did not report stunting prevalence. | \(n = 76\) Very young children 12 months. Recruited through child health centres. | Cohort study. Monthly interviews on feeding. Length and weight. **Intake:** Not reported. **Status:** Venous blood samples. All infants were well at sample collection. | Zinc intake  
Not reported.  
**Zinc status**  
Mean serum zinc: 11.5 μmol/L.  
Prevalence of low zinc status = 36% (cut off of <10.7 μmol/L). | Positive association: serum iron and TIBC.  
Negative association: sTfR. No association with growth measurements or infant sex. | • Did not assess dietary zinc intake.  
• Did not state whether strict zinc protocol was used for preventing contamination.  
• Did not use standard IZiNCG cut off for low zinc status. |
<table>
<thead>
<tr>
<th>Bouglé et al. (2000)</th>
<th><strong>France</strong> High income country. No (0%) stunting present.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n = 66</strong></td>
<td>Very young children &lt;3 years. Healthy children with normal growth, recruited through the hospital pediatric department.</td>
</tr>
<tr>
<td><strong>Cross-sectional.</strong></td>
<td>Height and weight.</td>
</tr>
<tr>
<td><strong>Intake:</strong> No reported.</td>
<td><strong>Zinc intake</strong> Not reported.</td>
</tr>
<tr>
<td><strong>Status:</strong> Blood sample.</td>
<td><strong>Zinc status</strong> Mean (SD) serum zinc: 14.2 (3.0) μmol/L. Prevalence of low zinc status (&lt;12 μmol/L) = 21%.</td>
</tr>
<tr>
<td>Excluded those with inflammation, defined as CRP &gt;10 mg/L.</td>
<td>No association between serum zinc concentrations and age. No association between growth rate (height gain and weight gain) and serum zinc concentrations. Serum zinc concentrations were not related to any parameters of iron status (serum iron, transferrin, transferrin saturation, haemoglobin, hematocrit or MCV).</td>
</tr>
</tbody>
</table>

- Cross-sectional nature.
- Study was underpowered to assess the effect of serum zinc on growth (20% power).
- Blood was drawn from infants with suspected iron deficiency.
- Did not state the type of biochemical sample taken (venous or capillary) or if the blood sample was collected in trace-element free vacutainers.
- Did not assess dietary zinc intake.
- Did not use standard IZINC cut off for low zinc status.
Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)¹

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kattelmann et al. (2001)</td>
<td>United States</td>
<td>n = 175 (all data)</td>
<td>Randomised prospective trial. Randomised to either early introduction (3-4 months) (n=90) or late introduction (at 6 months) (n=82) of commercial or parent choice of complementary foods. Weight, length and head circumference. <strong>Intake:</strong> 3-day diet history at 3, 6, 12, 18, 24, 30 and 36 months as well as 1-day diet records at 4, 5, 7, 8, 9, 10 and 11 months. Homogenous to milk intakes – all FF after 3 months. <strong>Status:</strong> Fasted venous blood samples at 12, 24 and 36 months.</td>
<td>Zinc intake&lt;br&gt;Mean range between 3-36 months, mg/day:&lt;br&gt;Early: 4.1-5.9.&lt;br&gt;Later: 4.0-6.2.&lt;br&gt;Zinc status&lt;br&gt;Mean (range) plasma zinc, μmol/L:&lt;br&gt;At 12 months:&lt;br&gt;Early: 13.9 (10.1-18.4).&lt;br&gt;Later: 14.5 (10.6-22.2).&lt;br&gt;At 24 months:&lt;br&gt;Early: 12.4 (8.4-16.5).&lt;br&gt;Later: 12.2 (8.9-19.1).&lt;br&gt;At 36 months:&lt;br&gt;Early: 11.9 (8.3-18.4).&lt;br&gt;Later: 12.1 (7.8-23.3).&lt;br&gt;Prevalence of low zinc status (&lt;9.2 μmol/L) = 0% at 12 months, 3% at 24 months, and 2% early and 6% late at 36 months.</td>
<td>At 5 and 6 months of age early introduction of solids group consumed significantly less zinc than those who were in the group which introduced to solids later. Although this was not observed at any other time-point. There was no relationship between serum zinc concentration and dietary zinc intake.</td>
<td>• Several diet records collected (n=7 3-day, n=7 1-day), which may have impacted on accuracy due to participant burden. • Did not state whether strict zinc protocol was used for preventing contamination. • Serum zinc was analysed in approximately half of the samples n=31-45. • Only measured CRP - this measure does not identify chronic inflammation. • CRP was not determined in all samples due to small blood volume collected. • Did not use standard IZINC cut off for low zinc status.</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intake:</strong> 24hr recall. <strong>Status:</strong> Venous blood samples (from forearm). Excluded those with inflammation, defined as CRP $&gt;3$ mg/dL.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zinc intake</strong> Not reported. <strong>Zinc status</strong> Mean (95% CI) serum zinc (0.5-2 years)*: 10.6 (10.3, 10.9) μmol/L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence of low serum zinc was highest $&lt;$24 months, the prevalence declined with increasing age. Likelihood of having low serum zinc was lower as SES increased (used as a continuous variable). Higher SES lead to lower risk of low serum zinc. No other associations with zinc status were reported in children. SES was the only significant predictor of zinc intakes in children.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Cross-sectional nature. • Authors split children into age ranges, however, only a small sample was within the group of interest (0.5-2 years, $n=31$). • Did not report actual mean zinc intakes of the groups. • Did not state whether strict zinc protocol was used for preventing contamination.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
</table>
| Taylor et al. (2004) | United Kingdom | *n* = 198 Very young children aged 4-24 months. Recruited before 4 months of age from middle class population. | Longitudinal prospective study. **Intake:** 7-day WDR at 4, 8, 12, 16, 20 and 24 months. Parents chose how much meat to offer their child – this was used to categorise them into ‘non-meat eaters’, ‘mixed (red and white) meat eaters’ – given 3 tertiles. **Status:** Capillary blood samples at 4, 12 and 24 months. - Used trace-element free techniques. | Zinc intake  
Mean (SD), mg/day:  
At 8 months: 3.9 (1.5) non-meat, 4.3-4.6 (1.4-1.6) meat eaters.  
At 12 months: 5.0 (1.8) non-meat, 4.7-4.9 (1.2-1.5) meat eaters.  
Zinc status  
Mean (SD) plasma zinc, μmol/L:  
At 4-5 months: 11.7 (1.1) non-meat, 12.9-14.2 (2.0-3.5) meat eaters.  
At 12 months: 13.6 (2.2) non-meat, 14.0-15.4 (2.6-3.3) meat eaters.  
Prevalence of low zinc status (<11 μmol/L) = 7.4% at 12 months. | No association between serum zinc across diet groups at any age.  
No association between meat intake and zinc status.  
No association between total zinc intake and risk of zinc deficiency.  
Did not look at any other associations. | - Was not specified where infants were recruited from.  
- Multiple (*n*=6) 7-day WDRs collected - this may have impacted on accuracy due to participant burden.  
- Meat group was split into tertiles - small numbers in meat group for status (range, *n*=18-36) and intake (*n*=32-49), and non-meat group for status (*n*=8-15) and intake (*n*=11-15).  
- Capillary blood used for zinc status – may result in lower zinc concentrations.  
- Did not adjust for inflammation.  
- Did not use standard IZiNCG cut off for low zinc status. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Study Design</th>
<th>Sample Size</th>
<th>Study Details</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torrejón et al. (2004)</td>
<td>Chile</td>
<td>Cross-sectional.</td>
<td>n = 42</td>
<td>Male toddlers 18 months. Recruited through child wellness clinic. Cross-sectional. Weight, length, tricipital and subscapular skinfolds. WHO standards for z-score calculation. <strong>Intake:</strong> 24hr diet history. <strong>Status:</strong> Fasted venous blood sample. -Used trace-element free techniques.</td>
<td>Zinc intake Mean (SD): 5.2 (1.9) mg/day. Zinc status Mean (SD) plasma zinc: 12.7 (1.9) μmol/L. Prevalence of low zinc (&lt;12.3 μmol/L) = 55%. Association between plasma zinc and weight-for-length ratio, zinc intake, and subscapular skinfold.</td>
</tr>
<tr>
<td>Morgan et al. (2010)</td>
<td>New Zealand</td>
<td>Randomised placebo controlled trial.</td>
<td>n = 225 (all data)</td>
<td>Toddlers 12-20 months. Recruited through newspaper advertisements, flyers, letters sent to families through newspaper birth notices. Randomised placebo controlled trial. Randomised into meat group n=90, fortified toddler milk group n=45, or non-fortified milk placebo group n=90. Demographic questionnaire. Weight and length. <strong>Intake:</strong> 3-day WDR at baseline, week 4 and week 18. <strong>Status:</strong> Non-fasting venous blood samples. -Used trace-element free techniques.</td>
<td>Zinc intake Mean (SD) at baseline, mg/day: Placebo group: 5.2 (1.3), meat group: 5.0 (0.8), fortified milk group: 4.6 (1.2). Zinc status Geometric mean (95% CI) serum zinc at baseline, μmol/L: Placebo group: 9.8 (9.5, 10.2), meat group: 9.5 (9.2, 9.8), fortified milk group: 10.0 (9.4, 10.5). Prevalence of low zinc status = 30-48%. Intervention improved zinc intakes of non-BF toddlers in both the meat and fortified milk groups from baseline to week 4. Significant increase in serum zinc in the fortified milk group from baseline to end of intervention but not in the meat group. Post hoc analysis found no significant association between red meat intake or other meat intake and serum zinc status.</td>
</tr>
</tbody>
</table>
Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)\(^1\)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country(^2)</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status (^3)</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Han et al. (2011)</td>
<td>Korea</td>
<td>n = 51</td>
<td>Cross-sectional.</td>
<td>Zinc intake</td>
<td>Positive association between</td>
<td>• Cross-sectional nature.</td>
</tr>
<tr>
<td></td>
<td>High income</td>
<td>Very young</td>
<td>Data collected at 1, 2, 4,</td>
<td>Mean (SD), mg/day:</td>
<td>HM consumption and serum</td>
<td>• Small sample size.</td>
</tr>
<tr>
<td></td>
<td>country.</td>
<td>children 0-36</td>
<td>5, 6, 12, 18, 24 and 36</td>
<td>At 5 months:</td>
<td>zinc concentrations at 5</td>
<td>• Did not specify if trace-element free</td>
</tr>
<tr>
<td></td>
<td>Did not</td>
<td>months.</td>
<td>months.</td>
<td>1.0 (0.8) HM.</td>
<td>months.</td>
<td>vacutainers were used – although stated maximum caution was</td>
</tr>
<tr>
<td></td>
<td>report</td>
<td>Mothers</td>
<td>Weight and height.</td>
<td>3.8 (0.7) CBF.</td>
<td>No other associations were</td>
<td>taking during blood collection to prevent</td>
</tr>
<tr>
<td></td>
<td>stunting</td>
<td>recruited in</td>
<td>Mothers chose a type</td>
<td>7.0 (1.5) SBF.</td>
<td>seen between zinc intake</td>
<td>contamination.</td>
</tr>
<tr>
<td></td>
<td>prevalence.</td>
<td>third</td>
<td>of infant milk: human</td>
<td>At 12 months:</td>
<td>and serum zinc in all groups</td>
<td>• Use of 24h diet recall may have resulted in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trimester</td>
<td>milk (HM), casein-based</td>
<td>5.7 (3.2) HM.</td>
<td>at 5, 12 or 36 months.</td>
<td>memory lapses of what was actually consumed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>through local</td>
<td>formula (CBF) and soy-</td>
<td>5.8 (1.3) CBF.</td>
<td>• Table 3 – serum zinc</td>
<td>• Table 3 – serum zinc concentrations reported as mg/day – this is an error as</td>
</tr>
<tr>
<td></td>
<td></td>
<td>parenting</td>
<td>based formula (SBF), to</td>
<td>11.6 (3.0) SBF.</td>
<td>concentrations reported as</td>
<td>they have used the reference of 9.9 μmol/L for low serum zinc so should be</td>
</tr>
<tr>
<td></td>
<td></td>
<td>programmes</td>
<td>use for first 5 months.</td>
<td></td>
<td>μmol/L.</td>
<td>μmol/L.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and maternity</td>
<td>Test weighing used for</td>
<td></td>
<td></td>
<td>• Did not report prevalence of low zinc status at 12 months.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hospitals.</td>
<td>HM intakes.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intake: Dietary zinc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>intake assessed at 1, 2,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4, 5, 6, 9, 12, 18, 24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and 36 months.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24h diet records used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>for assessing solid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>food intake.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Status: Venous blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sample at 5, 12 and 36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>months.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenault et al. (2011)</td>
<td>Peru and Ecuador</td>
<td>Upper middle income countries. Mild-to-moderate level of stunting.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------</td>
<td>---------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peru infants 6-8 months (n=297).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ecuador very young children 12-30 months (n = 579). Recruit as random sample and through house-to-house and health centres.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Randomised controlled trial (only pre-intervention data reported). SES data collected as an index based on primary housing quality factors ranked 0-10.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intake: Not reported.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Status: Venous blood sample – in well infants only.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc intake</td>
<td>Not reported.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc status</td>
<td>Mean (SD) plasma zinc, μmol/L*: Peru: 11.9 (2.2), Ecuador: 11.2 (2.2).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Peru - negative associations: time of day of blood sampling, CRP.
- Peru - positive associations: number of hours since previous meal, SES.
- Ecuador - negative associations: time of day of blood sampling, elevated CRP.
- Ecuador - positive associations: SES.

- Infants had low length-for-age z-scores at baseline.
- Includes two different studies, which makes interpretation difficult.
- Did not assess dietary zinc intake.
Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)¹

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country³</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status ³</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarwal et al. (2013)</td>
<td>Delhi, India Lower middle income country. Did not report stunting.</td>
<td>n = 339 (all data)Bo, &lt;2,500 g (n=220). NBW (n=119). Recruited through three hospitals in Delhi, all healthy live born infants were included.</td>
<td>Cross-sectional. <strong>Intake:</strong> Baseline questionnaire, including food habits, supplementation. All infants were followed up at 14 weeks corrected age. <strong>Status:</strong> Venous blood sample of infant at birth and follow up. -Used trace-element free techniques.</td>
<td><strong>Zinc intake</strong> Not reported. <strong>Zinc status</strong> Median (IQR) serum zinc, μmol/L*: At <em>birth</em>: LBW: 9.8 (5.0-15.4). NBW: 10.3 (6.8-20.9). At <em>follow up</em>: LBW: 8.2 (4.6-15.8). NBW: 9.2 (6.4-12.8). Prevalence of low zinc status = 52% LBW and 43% NBW at birth, 79% LBW and 67% NBW at follow up.</td>
<td>Serum zinc concentrations significantly higher in NBW infants at follow up (14 weeks) compared with LBW infants. No association between maternal and infant zinc status at birth.</td>
<td>• Cross-sectional nature. • Biochemical zinc was not analysed for all samples (those with results both at birth and follow up, n=100 LBW and n=66 NBW). • Did not report dietary zinc intakes. • Included infants born pre-term.</td>
</tr>
</tbody>
</table>
Engle-Stone et al. (2014) Cameroon, Africa Lower middle income country. High (33%) level of stunting.

$n = 882$
Toddlers and pre-school children aged 12-59 months. Recruited from random households.

Cross-sectional.
Length (children <2 years) or height, weight.

**Intake:** 24h diet recall.

**Status:** Venous blood samples.
-Used trace-element free techniques.

**Zinc intake**
Mean zinc intake, mg/day:
BF children: 1.6-2.4,
Non-BF children: 3.6-5.7.

Mean phytate intake, mg/day:
BF children: 210-290,

**Zinc status**
Range of mean plasma zinc (Table 3)*:
Children: 7.3-8.7 μmol/L.

Unadjusted values above - adjusted PZC slightly higher.
Prevalence of low zinc status = >70%.

**Negative associations:** time since last meal, CRP, AGP, and appeared to be negatively associated to dietary zinc intake.

**Risk factors for low plasma zinc in children:** residing in rural areas, stunting, low SES, low weight-for-height z-score, and low maternal education.

- Cross-sectional nature.
- Use of 24h diet recall may have resulted in memory lapses of what was actually consumed.
- Data collected from different regions – dietary intakes across these regions may differ which has not been reported.
Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)\(^1\)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country(^2)</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status(^3)</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelova et al. (2014)</td>
<td>Bulgaria Upper middle income country. Ukraine Lower middle income country. Did not report stunting prevalence.</td>
<td>(n = 103) (all data) Infants and toddlers (0-3 years) with IDA. Recruited through hospitals in study countries.</td>
<td>Cross-sectional. Split into 3 groups, Group 1 – Bulgarian with IDA ((n=30)), Group 2 – Ukrainian with IDA ((n=48)), Control group ((n=25)). <strong>Intake:</strong> Parental questionnaire regarding feeding pattern. <strong>Status:</strong> Morning fasting venous blood sample. -Used trace-element free techniques.</td>
<td>Zinc intake Not reported. <strong>Zinc status</strong> Mean (SD) serum zinc, (\mu)mol/L: Group 1 with IDA: 11.2 (4.4). Group 2 with IDA: 11.0 (1.8). Control group: 18.0 (1.1).</td>
<td>Serum zinc was lower in infants with IDA compared with Control infants.</td>
<td>• Cross-sectional nature. • Did not report dietary zinc intakes. • Did not assess inflammation or infection.</td>
</tr>
</tbody>
</table>
| Cantoral et al. (2015) | Mexico Upper middle income country. Did not report stunting prevalence. | Cross-sectional. Weight and length. **Intake:** Validated semi quantitative FFQ. **Status:** Morning fasting venous blood sample. | **Zinc intake**
Mean (SD or IQR), mg/day:
Zinc: 8.0 (2.3).
Phytate: 545 (190).
Phytate:zinc molar ratio: 6.9 (5.4-8.4).
**Zinc status**
Mean (SD) serum zinc*: 12.2 (2.5) μmol/L.
Prevalence of low zinc status = 17%.
No association between serum zinc concentrations and sex. Total dietary zinc (mg/day) was not a significant predictor of serum zinc. |
|---|---|---|---|
| n = 333 Children aged 2 years. Recruited as they were affiliated with the Social Security Institute in Mexico. | | | • Cross-sectional nature.
• Used an FFQ to assess dietary intake - may have overestimated intake due to memory lapses of actual quantity consumed.
• Did not state whether strict zinc protocol was used for preventing contamination.
• Did not assess inflammation or infection. |
### Table 2.8 (continued) Studies reporting biochemical zinc status with- or without intake data in very young children without known disease (from year 1993 onwards)¹

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country¹</th>
<th>Participants</th>
<th>Design/methods</th>
<th>Zinc intake and status³</th>
<th>Associations with zinc status</th>
<th>Study limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galetti et al. (2016)</td>
<td>Benin, Africa Low income country. Very high (51%) level of stunting.</td>
<td>n = 598 (all data) Preschool aged children 1–5 years (n = 326). Recruited from a rural community, no access to improved water. School-age children 5–10 years (n = 272). Recruited from a rural community with basic infrastructure and improved water.</td>
<td>Cross-sectional. Purpose was to examine feasibility of delivering zinc via drinking water. Height, weight and mid upper arm circumference. <strong>Intake:</strong> 3DDR. <strong>Status:</strong> Venous blood samples. -Used trace-element free techniques.</td>
<td><strong>Zinc intake</strong>&lt;br&gt;Mean: Zinc: 8.1 mg/day. Phytate: 1.69 g/day. Phytate-to-zinc molar ratio: 22.2. Prevalence of inadequate intakes = 10.5% (IZiNCG). <strong>Zinc status</strong>&lt;br&gt;Median (IQR) plasma zinc, μmol/L*: Preschoolers: 8.9 (7.5, 10.2). School-age: 10.5 (9.3, 11.5). Prevalence of low zinc status = 55% and 34% (CRP adjustment).</td>
<td>Independent predictors of plasma zinc status: Pooled data: age, no formal education of guardian vs formal education, farming as work vs other work. High CRP, non-fasting. Preschool: High CRP, not fasting, farming as work vs other work. School-age: AGP, no formal education of guardian vs formal education.</td>
<td>• Cross-sectional nature. • 3DDR data was collected from a subsample (n=36, 13%) of older children (5-11 years).</td>
</tr>
</tbody>
</table>

**Abbreviations:** 3DDR, three day weighed diet record; AGP, α₁-acid glycoprotein; BF, breastfed; BM, breast milk; CBF, casein-based formula; CRP, C-reactive protein; d, day; EBF, exclusive breastfeeding; FFQ, food frequency questionnaire; HAZ, height-for-age z-score; HM, human milk; IDA, iron deficiency anaemia; IF, infant formula; IGF-1, insulin like growth factor-1; IQR, Interquartile range; IZiNCG, International Zinc Nutrition Consultative Group; LAZ,
length-for-age z score; MCV, mean cell volume; NE, North East; NZEO, New Zealand European and other; PZC, plasma zinc concentration; SBF, soy-based formula; SES, socio-economic status; sTfR, soluble transferrin receptor; WAZ, weight-for-age z-score.

1 Limited to studies in healthy children. Excludes trials where infants were supplemented with zinc, unless the trial reported results at baseline or was an intervention of food-based strategies. No review studies or meta-analyses were included in the table.

2 Income level defined based on the World Bank Ranking list of economies July 2016 (The World Bank, 2016). Stunting level based on prevalence estimates by the WHO as <20%: low, 20-29.9%: moderate, 30-39.9%: high, ≥40: very high (Gorstein et al., 1994).

3 Prevalence of low zinc status defined in the study using the reference level of <9.9 μmol/L recommended by IZiNCG (2007), unless stated otherwise.

* Study reported zinc status as μg/dL, which was converted to μmol/L by dividing by 6.54.

** Study reported zinc status as mg/L, which was converted to μg/L (1 mg/L = 100 μg/L), then divided by 6.54 to get μmol/L.
2.8 Conclusion

Despite increased interest in BLW as an alternative approach to complementary feeding, there is very little research on BLW, in particular research investigating the concerns expressed by health professionals regarding the potential increased risk of iron (and therefore zinc) deficiency, growth faltering, and choking. To date, only one study has assessed the nutrient intakes of infants following BLW, but no study has assessed the biochemical nutrient status of these infants.

Determining the nutrient intakes and status of very young children can be challenging due to having to rely on caregivers to provide accurate dietary intake data, and to obtain biochemical samples from very young children. This is difficult due to their higher percentage of body fat which makes it difficult to find their veins, and to their dislike of being restrained during sample collection.

Although only few studies have assessed iron and zinc intakes and status of very young children in NZ, iron intakes appear adequate for infants, although somewhat inadequate for toddlers, but the prevalence of iron deficiency and iron deficiency anaemia is relatively high. Zinc intakes of very young children in NZ appear adequate, however, their zinc status appears borderline, although very few studies have reported zinc status in NZ infants and toddlers. Given the apparent high rates of iron and zinc deficiency amongst very young children following traditional feeding practices, it is particularly important to determine the impact of baby-led approaches to complementary feeding. There is also very little information on potentially modifiable ‘predictors’ of zinc status in this age group in high-income countries such as NZ.
2.9 Aim and objectives of this thesis

Therefore, the aim of this thesis was to determine the impact of this version of BLW modified to prevent iron deficiency, on iron and zinc intakes and status, and to determine potentially modifiable ‘predictors’ of zinc status in toddlers. The specific objectives were to:

1. Determine the iron intake (at 7 and 12 months of age) and iron status (at 12 months of age) of infants following BLISS compared with those of infants following traditional spoon-feeding (Chapter 4).

2. Determine the zinc intake (at 7 and 12 months of age) and zinc status (at 12 months of age) of infants following BLISS compared with those of infants following traditional spoon-feeding (Chapter 5).

3. Examine associations between biochemical, dietary, and other variables, and plasma zinc concentration, and to determine potentially modifiable ‘predictors’ of zinc status at 12 months of age (Chapter 6).
3 Methods

This chapter describes the methods for the BLISS study that are relevant to this thesis.

3.1 Study design and participants

The Baby-Led Introduction to SolidS (BLISS) Study was a 2-arm randomised controlled trial (Figure 3.1), commencing in late pregnancy. All pregnant women booked into the Queen Mary Maternity Unit, Dunedin Hospital (Dunedin, New Zealand), were invited to participate in the BLISS study during their third trimester of pregnancy. There are no other birthing facilities in Dunedin (population approximately 120,000) and the number of home births is <3%. Each woman received a letter that acknowledged their booking into the maternity unit and provided them with initial information about the study. Women who requested home births were given similar information regarding the study from their Lead Maternity Carer (LMC). All mothers in New Zealand choose an LMC, usually a midwife, who is responsible for their pregnancy-related health care from pregnancy to approximately 6 weeks after birth. Just before 28 weeks gestation, the prospective participant’s LMC was contacted to ensure that invitation letters were not sent to women who had miscarried. At 28 weeks gestation, the prospective participant received a letter inviting them to take part in the study. This letter contained an opt-out phone number for an answerphone where the woman could leave a message advising if they did not wish to participate. Women who did not opt-out within two weeks of invitation into the study were contacted by research staff to establish eligibility, explain the purpose of the study, answer any questions and, if they were interested in participating, organise a time for an individual meeting so that the woman could give written informed consent to participate.
Written informed consent was obtained from all participants before randomisation. The study was approved by the Lower South Regional Ethics Committee (LRS/11/09/037) and is registered with the Australian New Zealand Clinical Trials Registry ACTRN12612001133820.

Figure 3.1 CONSORT diagram of the BLISS study participants, as relevant to the analysis of iron and zinc intake and status
Women were eligible to participate if they: booked into the birthing unit at Queen Mary Maternity Hospital before 34 weeks gestation (those women who had chosen a home birth were considered eligible if their midwife notified the study before 34 weeks gestation); spoke English or Te Reo Māori (the official language of the indigenous people of New Zealand); planned to live in the Dunedin, New Zealand, area until their child was at least two years of age; and were 16 years of age or older. After birth, women were excluded if their infant was born before 37 weeks gestation; or if a congenital abnormality, physical condition, or intellectual disability which was likely to affect the infant’s feeding or growth was identified.

3.2 Sample size

A total of 84 participants per group were required to detect a difference in geometric mean plasma ferritin concentration of 5.0 μg/L with 80% power (α=0.05) (Daniels et al., 2015). Allowing for a 15% dropout, this was equivalent to a sample size of 198 participants.

3.3 Randomisation

All eligible participants were randomised into one of the two study groups (Control or BLISS) after stratification. The study biostatistician used numbers from random length blocks to create four strata for parity (1st child vs >1 child) and education (non-tertiary vs tertiary), as these may have affected responsiveness to the intervention:

1. Non-tertiary education and 1st child
2. Tertiary education and 1st child
3. Non-tertiary education and >1 child
4. Tertiary education and >1 child

Once this information was collected in the baseline questionnaire (Appendix A, Question 9: answers 1-4 = non-tertiary, answers 5-8 = tertiary, and Question 26: answer 1 = 1st child, answers 2-4 = >1 child), research staff opened the next
consecutive opaque, pre-sealed envelope in the stratum to which the participant belonged and then informed the participant which group they had been assigned to.

All outcome assessment data were collected by research staff who were blinded to group allocation.

3.4 Study groups and intervention

All participants received routine midwifery (until 6 weeks of age) and Well Child (from 6 weeks of age) care. Well Child Tamariki Ora is a nationally funded health care programme for all children under 5 years of age within New Zealand (Ministry of Health, 2014). The programme involves free home and clinic visits by community-based nurses who provide advice on feeding, sleep and safety; and assess growth and development, hearing, vision, and wellness. The 10 visits up to 5 years of age are typically scheduled for: birth, 1 week, 2-4 weeks, 4-6 weeks, 8-10 weeks, 3-4 months, 5-7 months, 9-12 months, 15-18 months, and 2-3 years (Ministry of Health, 2014).

3.4.1 Control

Participants randomised to the Control group received routine care (as described above) from the providers of their choice, and no additional intervention.

3.4.2 BLISS

Participants randomised to the BLISS group received routine care (as described above) from the provider of their choice, as well as additional parent contacts for support and education from before birth to 9 months of age delivered by the BLISS study. The intervention was delivered by BLISS staff: an experienced International Board Certified Lactation Consultant (IBCLC), and trained research staff who were supervised by a multidisciplinary team (dietitian, paediatrician, speech-language therapist) throughout the study. The
intervention had three key components: contact with an IBCLC, BLISS advice, and BLISS resources.

**IBCLC (third trimester of pregnancy to 6 months of age)**

There were five or more contacts with an IBCLC:

1. An anticipatory guidance group session before birth (at approximately 34-35 weeks gestation): to discuss breastfeeding (benefits, challenges and developing a “breastfeeding plan”), explain the nature of the free support service offered until their infant was 6 months of age, and introduce the concept of BLISS.

2. A home visit in the first week after the mother returned home from hospital, or during the first week following a planned homebirth; a support phone call and offer of a home visit at 3-4 weeks; a home visit at 3-4 months; and a phone call at 5 months of age: to provide support and education on breastfeeding (or formula feeding if requested), and to assess how the advice of offering milk only until six months of age was going. Support included encouraging: exclusive breastfeeding to 6 months, breastfeeding to at least 12 months, and delaying the introduction of complementary foods until 6 months of age.

3. The lactation consultant was also available to supply additional support when requested by the participant until her infant was 6 months of age: this involved providing specific individualised advice to address problems with breastfeeding (or formula feeding) via extra home visit(s), phone or email contact. This additional support was utilised by 36% of families (Davies, personal communication) in the earlier Prevention of Overweight in Infancy study in the same city (Taylor et al., 2011).

**BLISS advice (5.5 to 9 months of age)**

There were at least three contacts with BLISS research staff. Home visits at 5.5, 7 and 9 months of age were provided to give individualised advice and support for the introduction of complementary foods using the BLISS approach. Parent
participants were advised that they must not start BLISS until their infant was 180 days (i.e. 6 months of age). Research staff encouraged responsive feeding (Black et al., 2011), particularly advising that: the infant was not to be distracted while eating, caregivers were to pay attention to the infant’s hunger and satiety cues, and the caregivers responded to the infant promptly and supportively. Parents were encouraged to offer “easy” foods and more frequent milk feeds during both illness and recovery (World Health Organization, 2004b). The research staff were also available to provide additional support when requested by the participant.

Parents were encouraged to offer three food types at each meal:

1. An iron-rich food (e.g., red meat, iron-fortified infant cereal).
2. An energy-rich food (>1.5 kcal/g, e.g., avocado, cheese).
3. An easy food (e.g., fruit or vegetable).

A range of resources were given to participants explaining how to follow BLISS and providing age-appropriate family recipes (see below).

**BLISS resources (third trimester of pregnancy to 9 months of age)**

A range of resources developed and pretested for the purposes of this study were provided to the participants, including information about the BLISS study, recipe books, everyday food lists, and safety information (Cameron et al., 2015). These resources followed the philosophy of BLW but also addressed the three key concerns that health professionals had expressed about BLW (Cameron et al., 2012b; D’Andrea et al., 2016): inadequate iron intake, growth faltering, and choking. All resources were developed in conjunction with a paediatric speech-language therapist to address concerns about choking. In particular, the resources encouraged parents to:

1. Test foods before they were offered to ensure they were soft enough to mash with the tongue on the roof of the mouth (or were large and fibrous enough that small pieces did not break off when sucked and chewed, e.g., strips of meat).
2. Avoid offering foods that formed a crumb in the mouth.
3. Offer foods that were at least as long as the child’s fist, on at least one side of the food.

4. Ensure the infant was always sitting upright when they were eating – never leaning backwards.

5. Always have an adult with the child when they were eating.

6. Never put whole foods into the infant’s mouth – the infant was to do this at their own pace and under their own control.

Resources were used at different visits to accompany the advice given by the BLISS study IBCLC and research staff: ante-natal (n=1), 3-4 months (n=1), 5.5 months (n=6), 7 months (n=2) and 9 months (n=1) (Figure 3.2). Resources included information on when to start complementary feeding, safety advice, BLISS in a nutshell (Appendix B), recipe books, and laminated sheets of example foods to offer including the three types of foods to offer at every meal – an iron-rich food, an energy-rich food, and an easy food (Appendix C). The BLISS participants were also provided with three complementary packets of iron-fortified infant cereal (Heinz-Watties For Baby Rice Cereal, Heinz-Watties Ltd., Australia) at each of the intervention visits (5.5, 7, and 9 months). The iron content of this infant cereal was 2.2 mg per 100 g of infant cereal prepared with water, this product was not fortified with zinc.
<table>
<thead>
<tr>
<th>Time point</th>
<th>34-35 wk gestation</th>
<th>1wk</th>
<th>3-4 wk</th>
<th>2mo</th>
<th>3mo</th>
<th>4mo</th>
<th>5mo</th>
<th>5.5mo</th>
<th>6mo</th>
<th>7mo</th>
<th>8mo</th>
<th>9mo</th>
<th>12mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactation advice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BLISS advice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Feeding Questionnaire (phone)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breastfeeding status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extent of spoon and puree feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gagging and choking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weighed diet record</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Key questionnaires</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy self-regulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental feeding behaviour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acceptability and cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gagging and choking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood collection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-.BLISS (intervention) participants receive this
- BLISS (intervention) and usual-care (control) participants receive this

**Figure 3.2** Intervention and outcome measures at specific time points (as published in Daniels et al. (2015))
3.5 Adherence

Questionnaires at 2, 4, 6, 7, 8, 9 and 12 months of age were used to determine adherence to BLISS by asking parents at each time point - ‘how has your baby been fed solids in the past week?’, with responses: 1) fed by an adult, 2) mostly fed by an adult and some baby fed themselves, 3) about half spoon-fed by an adult and half baby fed themselves, 4) mostly baby fed themselves and some fed by an adult, or 5) baby fed themselves. Adherence to BLISS was defined as the infant feeding themselves most or all of their food in the past week at 7, 8, 9, and 12 months of age (Appendix D: Question 21, response 4 or 5).

3.6 Questionnaire data

Demographic data including: maternal age, ethnicity, education, and parity were collected at baseline by questionnaire (Appendix A). The participant's current address was used to determine the New Zealand Index of Deprivation (NZDep) score for each household (Atkinson et al., 2014). Information including infant sex, birth weight, and gestational age at birth was accessed through hospital records. At 2, 4, 6, 7, 8, 9 and 12 months of age brief feeding questionnaires were administered by research staff to collect information on: whether the infant was breast, or formula fed, at what age breastfeeding stopped and/or formula feeding started and stopped, and at what age 'high-iron' foods were first offered (iron-fortified cereal, red meat, other meat) (Appendix B). Parent participants completed a questionnaire (self-administered) at 12 months of age which included questions from the Children’s Eating Behaviour Questionnaire regarding food fussiness (Wardle et al., 2001). Parents answered questions using a 5-point Likert scale (with options: “never”, “rarely”, “sometimes”, “often”, or “always”) on six statements for determining food fussiness: enjoyment of tasting new foods, enjoying a wide variety of foods, interest in tasting new foods, refusing new foods, deciding whether a food is disliked before tasting, and whether there is difficulty in pleasing the infant with meals (Cronbach $\alpha=0.64$-
0.88) (Appendix E: Questions 46, 49, 55, 63, 71, and 72). Higher mean scores represent higher levels of food fussiness (Wardle et al., 2001).

### 3.7 Anthropometric assessment

Research staff measured infant weight (Seca, Model 334, Hamburg, Germany) and length (Rollameter 100c length board, Harlow Healthcare, United Kingdom) when they were 12 months of age. These measures were used to calculate weight-for-age and length-for-age z-scores using the WHO child growth standards reference data (de Onis et al., 2006), and to then determine whether any participants were underweight (using the weight-for-age z-score < -2 SD) or stunted (using the length-for-age z-score < -2 SD) (Gorstein et al., 1994).

### 3.8 Dietary assessment

Weighed three-day diet records (WDRs) were used to assess dietary intake at 7 and 12 months of age (Appendix F). Parent participants were given detailed written and oral instructions for completing the WDR and then recorded three randomly assigned non-consecutive days (two week days and one weekend day) over a three-week period. Each day of the week was represented approximately an equal number of times among participants to control for day-of-the-week effects. Dietary scales (Salter Electronic, Salter Housewares Ltd. Tonbridge, United Kingdom), accurate to ± 1 g were given to each participant to complete the WDR. The diet record had four key components:

1. The **diet record**, where information was recorded regarding the time of the day, type and brand of the food or drink, preparation method and consistency of the food or drink (puréed, mashed, diced or whole). This also included a question on ‘who fed the child’ (parent, child or both). The total weight of the food or drink was recorded both before offering the food or drink, and also after the infant had finished eating or drinking (scraping leftovers off the floor, baby, and the tray, and also ensuring animals stayed away from feeding times). This enabled the calculation of
leftovers by subtracting the amount leftover from the amount consumed (Appendix G).

2. A description of any recipes used, including the raw amounts of ingredients, the cooking method and proportion of the total recipe fed to the child.

3. An end of day questionnaire, to determine whether it was a typical eating day for the child and how the meals compared to those consumed by the rest of the family (ingredients and preparation method).

4. Supplement use, to determine whether the child had consumed any iron or zinc containing supplements on that day. The question asked about type, brand and amount taken.

At the same time, parent participants were also provided with an ‘away from home booklet’, an adapted food diary for childcare, and a laminated example of a completed food diary day to refer back to when completing the WDR (Appendix F). A record was kept of the dates of the three days each participant had been asked to complete the WDR and a phone call was made to the participant after the first day of recording was completed to check everything had gone well with completing the diary and to answer any questions the participant had. Once the WDR had been collected, at the completion of the three days, research staff checked the WDR to ensure no information was missing. If any information was missing or unclear, research staff contacted the participant for clarification.

3.9 Dietary analysis

The completed WDRs were entered into Kai-culator (Version 1.13s, University of Otago, New Zealand), a dietary analysis software programme that includes dietary data: from the New Zealand Food Composition Database (FOODfiles 2010, Plant and Food Research) (Ministry of Health, 2010), for commonly consumed recipes collated in the 2008/09 New Zealand Adult Nutrition Survey (Ministry of Health et al., 2011), and for commercial infant foods collated by the
research team (Clouston, 2014). The Candidate entered into Kai-culator the recipes from the recipe books that had been given to BLISS participants so that these did not have to be entered individually each time they appeared in the participants’ WDRs. A protocol and template for entering WDRs into Kai-culator that had been developed by Masters Candidates working in the BLISS team (Appendix G), were used when research staff entered the WDRs. A code book was developed to assign rules (estimations, default foods and substitutions) to certain foods which were not available or had missing information, to maintain consistency when entering the WDRs. This was used and adapted by all research staff entering and checking WDRs, including the Candidate.

Once the WDRs had been entered, they were then checked for accuracy and to ensure consistency. A protocol developed by the Candidate was used for checking of all WDRs (Appendix H). All 7 month WDRs \((n=162)\) were checked by two registered dietitians and all 12 month WDRs \((n=143)\) were checked by the Candidate (also a registered dietitian).

### 3.9.1 Infant milk intake

Participants were asked to record infant milk intake (breast milk, or infant formula). As it was not possible to directly measure breast milk intake, participants were asked to record in the WDR every time their child was breastfed. Total breast milk intake was estimated to be 750 g per day at 7 months of age and 448 g per day at 12 months of age, based on a quadratic curve fitted to the breast milk volumes reported by Dewey et al. (1991a). These amounts were determined to be the most appropriate estimation of breast milk intakes based on previous literature, by a previous Masters Candidate for intakes at 7 months of age (Williams-Erickson, 2015), and by the current Candidate for intakes at 12 months of age (Appendix I). The iron and zinc content of breast milk was assumed to be 0.07 mg per 100 g and 0.3 mg per 100 g, respectively (Ministry of Health, 2010). If the infant was fed both breast milk and infant formula then the gram amount of infant formula consumed was subtracted from the estimated total breast milk intake (i.e. 750 g or 448 g per
day) and the remaining amount was assumed to be the amount of breast milk consumed by the infant.

### 3.9.2 Food group analysis

The Candidate developed 16 specific food groups to assess dietary intakes of nutrients by food group. The food groups were created to take into account their potential contribution to iron and zinc intakes. In total, 1,682 individual foods were reported to have been consumed by the infants in the WDRs at 7 and 12 months of age. Each of these individual foods was categorised by the Candidate into one of these pre-specified food groups shown in Table 3.1. The Candidate then developed a spreadsheet to collate the data needed in order to determine the nutrient contribution of each of these food groups (Appendix J). The spreadsheet was used to assign, to each individual food and drink consumed during the WDR, a food group and the amount of energy (kJ), iron (mg), zinc (mg) and selenium (mg) each 100 grams of the individual food or drink would contribute to that food group (based on the nutrient data in Kai-culator described in Section 3.9 above). The 1,682 foods included in this spreadsheet were foods and drinks either consumed as is, or within a recipe consumed by an infant.
Table 3.1 Individual foods included in each food group for food group analysis

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Foods Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breads and cereals</td>
<td>Plain crackers (cruskits, corn crispbread, cream crackers, rice crackers, wholemeal crackers, rice cakes, cheese crackers etc.), english muffins, bread buns, bread (white, wholemeal, multigrain, rye etc.), pizza bases, cereals (Weetbix, cornflakes, rice bubbles, porridge, oats, Special K, Sultana bran etc.), noodles (udon, egg, rice etc.), rice, pasta, couscous, quinoa, chia, scones, waffles, pikelets, pancakes, popcorn, rice pudding, tapioca pudding, pastry sheets, rusks, spaghetti in tomato sauce.</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>Cow’s milk (including milk powder).</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>Baby rice cereal, baby muesli, baby porridge, iron-fortified teething rusks.</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>Apple, banana, orange, mandarin, kiwifruit, mango, melon, grapes, pears, apple sauce, berries (strawberries, blueberries etc.), fruit juice. All fruit - fresh, canned, stewed, poached, dried, juiced.</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Potato (mashed, includes home cooked chips etc.), kumara, pumpkin, carrot, green beans, peas, corn, capsicum, broccoli, cauliflower, corgette, mushroom, green leafy vegetables, mixed vegetables, avocado, tomatoes (fresh, canned, pureéd, paste), olives, vegetable soup. All vegetables - frozen, fresh, canned, in brine.</td>
</tr>
<tr>
<td>Dairy</td>
<td>Butter, cheese, yoghurt, sour cream, custard, ice cream, cream.</td>
</tr>
<tr>
<td>Legumes</td>
<td>Beans (lentils, chickpeas, kidney beans, baked beans), hummus, tofu, tempeh.</td>
</tr>
<tr>
<td>Nuts and seeds</td>
<td>All nuts and seeds, nut butter’s, tahini.</td>
</tr>
<tr>
<td>Eggs</td>
<td>Eggs (boiled, scrambled, poached etc.).</td>
</tr>
<tr>
<td>Red meat</td>
<td>Beef, lamb, mutton, veal, venison, red meat offal (beef kidney, lamb liver).</td>
</tr>
<tr>
<td>Fish and poultry</td>
<td>Fish (all types), poultry.</td>
</tr>
<tr>
<td>Other meat</td>
<td>Pork, processed meat (bacon, ham, salami, sausages, pastrami), chicken liver pate, meatloaf.</td>
</tr>
<tr>
<td>Category</td>
<td>Examples</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Beverages</td>
<td>Rice milk, soy milk, almond milk, coconut milk, raro juice, drinking chocolate, smoothies, tea.</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>All other foods. Fats (butter, oil, margarine), sauces and stocks (mayonnaise, dressings, gravy, white sauce, tomato sauce and relish, pesto, coconut cream, curry paste, pizza sauce), spreads (marmite, vegemite, jam, cheese spread, Nutella etc.), sweets (jelly, lollies, ice blocks, sorbet, milo, chocolate, maple syrup), baked goods (biscuits, croissants, cake, muffins, loaves, iced buns, pastries, waffle cones), fried foods (fries/potato chips, McDonalds, KFC), other foods including: cocoa, spices, herbs, vinegar, seaweed, coconut, muesli bars.</td>
</tr>
<tr>
<td>Breast milk</td>
<td>Breast milk.</td>
</tr>
<tr>
<td>Infant formula</td>
<td>All infant formulas.</td>
</tr>
</tbody>
</table>
Once the spreadsheet had been imported, Kai-culator was able to disaggregate recipes (allowing for moisture losses). Because Kai-culator was able to calculate the energy and nutrient amounts contributed by ingredients from each food group within a particular recipe, a ‘mixed dishes’ food group was not necessary. For example:

<table>
<thead>
<tr>
<th>BLISS 7 month recipe - Cheesy carrot and courgette slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients in the original recipe: 2 medium courgettes, 1 grated onion, 1 large grated carrot, 6 eggs, 1 cup flour, 1 teaspoon baking powder, and 1 cup grated cheese.</td>
</tr>
</tbody>
</table>

Calculating the amount of each key nutrient (e.g., iron) contributed by each food group in the portion actually consumed by the infant poses a number of challenges. First, each ingredient needed to be assigned to a food group, in this case this recipe included foods from four of the 16 food groups: ‘vegetables’ (courgette, onion, and carrot), ‘eggs’ (eggs), ‘breads and cereals’ (flour and baking powder), and ‘dairy’ (cheese). Second, the recipe needed to be adjusted to allow for moisture, and nutrient gains and losses. As the recipe ingredients were entered into Kai-culator in their raw form, a moisture retention factor (89%), cooking method (baked), cooking time (30 minutes), and temperature (200°C) needed to be added to allow Kai-culator to calculate the final total weight and available nutrients for the cooked recipe. A moisture retention factor was chosen from the United States Department of Agriculture nutrient retention factor database in Kai-culator; this particular recipe was assigned the moisture retention of quiche (89%). Third, the exact amount (g) of each of these food groups, as well as the amount of nutrients of interest: iron (mg), zinc (mg), and selenium (mg), that were consumed as a portion by the infant was calculated using data from the spreadsheet (Appendix J), which had been imported into Kai-culator.

As an example, **Table 3.2** demonstrates how the ingredients within the recipe above were split if a 100 g portion of this recipe was consumed.
Table 3.2 An example recipe showing the proportion of each food group and contribution to nutrient intakes for a 100 g portion of cooked cheesy carrot and courgette slice

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Amount (g)</th>
<th>Energy (kJ)</th>
<th>Iron (mg)</th>
<th>Zinc (mg)</th>
<th>Selenium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables</td>
<td>38.9</td>
<td>40.6</td>
<td>0.17</td>
<td>0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Eggs</td>
<td>34.1</td>
<td>195.2</td>
<td>0.63</td>
<td>0.36</td>
<td>8.47</td>
</tr>
<tr>
<td>Breads and cereals</td>
<td>14.3</td>
<td>208.9</td>
<td>0.20</td>
<td>0.20</td>
<td>4.07</td>
</tr>
<tr>
<td>Dairy</td>
<td>12.7</td>
<td>205.1</td>
<td>0.14</td>
<td>0.53</td>
<td>1.59</td>
</tr>
</tbody>
</table>

This method of assessing the contribution of recipes to individual food groups was particularly important as participants randomised to the BLISS group were asked to make recipes for their infant that included ‘high-iron’ foods such as meat, and iron-fortified infant cereal. As iron-fortified infant cereal is often consumed via a spoon (which is difficult for a young infant to self-feed) it was recommended that BLISS infants were offered this food within a recipe or as a spread on another food, for example spread on strips of toast. If we had used a ‘mixed dishes’ food group we would not have been able to assess exactly how much iron-fortified infant cereal was consumed by infants in the BLISS group as it would often have been just a component of this non-descriptive food group. This is also likely to have introduced bias, as it would have appeared that Control infants consumed more iron-fortified infant cereal than BLISS infants, whose iron-fortified infant cereal consumed in recipes would have been recorded as a ‘mixed dish’.

3.9.3 Nutrient analysis

Data on the key nutrients and food components of interest (energy, iron, zinc, phytate, and Vitamin C) were sourced from Kai-culator, which includes the food composition data described in Section 3.9. Values for amount consumed per day were exported from Kai-culator to be used in the statistical analysis.
3.9.4 Analysis of additional food components

Some data (specifically: ‘meat, fish, poultry’ (MFP), haem iron, non haem iron, and phytate) were not available in the New Zealand Food Composition Database (FOODfiles 2010, Plant and Food Research). Therefore, these data needed to be determined and imported into Kai-culator before dietary analysis. MFP, haem iron (estimating that 40% of the iron from meat was haem iron), and non-haem iron (the remaining iron content after haem iron was calculated) were calculated using a spreadsheet developed by a previous student (Barris, 2012). Phytate values were collated from the literature, and information from the manufacturers, by another student (Hartley, 2013).

To estimate the proportion of absorbable iron and zinc, the phytate-to-iron and phytate-to-zinc molar ratios were calculated. The molecular weights of phytate, iron and zinc are 660, 55.9 and 65.4 respectively (Brown et al., 2004). So the equations used were:

Phytate-to-iron molar ratio: \[
\frac{\text{phytate (mg) / 660}}{\text{iron (mg) / 55.9}}
\]

Phytate-to-zinc molar ratio: \[
\frac{\text{phytate (mg) / 660}}{\text{zinc (mg) / 65.4}}
\]
3.10 Blood sample collection

All parent participants were contacted when their baby was 11 months of age to book a blood test no earlier than 12 months of age, using a protocol developed by the Candidate (Appendix K). Parent participants were given an information kit prior to the blood test consisting of: an appointment card (Appendix L), pre-blood test instruction sheet (Appendix M), a tube of local anaesthetic (Ametop Gel, Smith & Nephew, Canada), and three plastic film dressings (OPSITE Flexgrid, Smith & Nephew, Canada). The day before the scheduled blood test appointment the Candidate contacted each parent participant to confirm the time of the appointment, complete an illness questionnaire (Appendix N), and explain the pre blood test instruction protocol including the time to apply the Ametop gel, and to give their baby a milk feed (Arsenault et al., 2003). As recent food intake affects plasma zinc concentrations (Arsenault et al., 2003) we asked parents to feed their baby milk (as much as they wanted of the milk they usually have, e.g., breast milk, formula, or cow’s milk) 90 minutes prior to the blood test appointment, and then to give no other food or drink until after the blood test. This was to standardise what was consumed prior to the blood test – milk was decided on as it is a food that all 12-month-old infants consume.

If the child was unwell the day before the scheduled blood test, as defined by the illness questionnaire (presence of fever, diarrhoea, or vomiting), the blood test was delayed for 14 days. If again in 14 days the infant was still unwell or had developed another illness the blood test was delayed again. Rescheduled visits were limited to three occasions and after this time the infant was booked in for the blood test even if unwell (as long as the parent consented).

A rigorous trace element-free protocol based on IZiNCG (Brown et al., 2004) recommendations and developed by the Candidate was used for non-fasting venous blood sample collection and analysis (Appendix O). Blood samples were drawn from an antecubital vein into a trace element-free lithium heparin anticoagulated tube (7.5 mL; Sarstedt S-Monовette, Nümbrecht, Germany). This particular Sarstedt S-Monovette tube was chosen because it allowed for the collection of only one tube of blood to determine multiple
indices (e.g., haemoglobin, plasma zinc and ferritin) and could be used as a syringe rather than a vacutainer, which was considered by our phlebotomist to be gentler on the infants’ small veins. A maximum of three blood draw attempts occurred to minimise infant and parent distress. During the blood test appointment, a checklist was completed (Appendix P) to: check the right participant was in attendance, check whether the Ametop gel had been applied and for the right amount of time, and record blood collection information to ensure standardisation of the blood collection methods. A zinc questionnaire was also completed to: ensure the pre-blood test protocol had been followed, determine if the infant had become unwell since the previous contact, and determine any use of topical zinc (Appendix Q). All participants were given a gift (book) at the completion of the blood test appointment to thank them for their contribution to this component of the study.

Of the 206 participants, blood samples were successfully obtained from 119 of the 146 infants whose parents consented to the blood test at 12 months of age (82%). Reasons for uncollected blood were: unsuccessful blood draw (n=26), refusal of the blood test after initial consent (n=26), withdrawal from the study (n=22). In addition, 13 participants could not be contacted or were living out of town. The reasons for unsuccessful blood draws were either because: the infant was unable to sit still, or the phlebotomist was unable to find the vein because of the high amount of body fat covering the vein.

### 3.11 Biochemical analysis

Collected tubes of whole blood were held in a cooler box post collection. The Candidate developed a post blood collection protocol (Appendix O) and blood processing chart for the required laboratory analysis (Figure 3.3). An aliquot of at least 1 mL whole blood was pipetted for the analysis of complete blood count and plasma ferritin (Section 3.11.1). The remaining whole blood was separated within two hours of sample collection (3500 rpm for five minutes) and aliquots of plasma were stored at -80°C until subsequent analysis (Figure 3.3).
Figure 3.3 Blood sample processing flowchart

Abbreviations: HN, Department of Human Nutrition; SCL, Southern Community Laboratories Ltd.
3.11.1 Iron analysis

Complete blood count (Sysmex XE 5000 automatic electronic analyser, Kobe, Japan) and plasma ferritin (Cobas 8000 unit e 602, Roche, United States (US)) were determined on the collection day by Southern Community Laboratories Ltd. (Dunedin, New Zealand), where external quality control measures are completed regularly.

Soluble transferrin receptor (sTfR) was determined using a Cobas C311 automatic electronic analyser (Roche, New Zealand), in the Department of Human Nutrition, University of Otago. The accuracy and precision of the analyses were checked using controls and in-house pooled samples (after every 20 samples). The analysed mean (SD, CV) values for the multilevel sTfR control (Roche Diagnostics, US) were 2.43 mg/L (0.02 mg/L, 1.0%) and 6.89 mg/L (0.08 mg/L, 1.1%), compared to manufacturers’ concentrations of 2.05 mg/L and 6.67 mg/L, respectively. The sTfR values were converted to be equivalent with the Flowers assay using the equation (Cogswell et al., 2009): 1.5 x Roche sTfR + 0.35 mg/L.

Body iron was calculated as the log ratio of sTfR to plasma ferritin concentration (Cook et al., 2003; Cogswell et al., 2009):

Body iron (mg/kg) = \frac{log_{10}(\frac{sTfR \times 1000}{ferritin} - 2.8229)}{0.1207}

Iron status categories were defined using ferritin, body iron, and haemoglobin as follows (Table 3.3). Of those considered iron sufficient, other anaemia was also determined (body iron ≥0 mg/kg and haemoglobin <110 g/L).
Table 3.3 Criteria used to determine the stage of iron deficiency

<table>
<thead>
<tr>
<th>Iron status category</th>
<th>Cut off value for each</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron sufficient</td>
<td>Plasma ferritin ≥15 μg/L(^a), in the absence of early functional iron deficiency, iron deficiency anaemia, and iron depletion</td>
</tr>
<tr>
<td>Iron depleted</td>
<td>Plasma ferritin &lt;15 μg/L(^a), in the absence of early functional iron deficiency, and iron deficiency anaemia</td>
</tr>
<tr>
<td>Early functional iron deficiency</td>
<td>Body iron &lt;0 mg/kg(^b) and haemoglobin ≥110 g/L(^c)</td>
</tr>
<tr>
<td>Iron deficiency anaemia</td>
<td>Body iron &lt;0 mg/kg(^b) and haemoglobin &lt;110 g/L(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Cut off value obtained from Southern Community Laboratories Ltd. (2012)  
\(^b\)Cut off value obtained from Cook et al. (2003)  
\(^c\)Cut off value obtained from the World Health Organization (2001)

3.11.2 Zinc analysis

Plasma zinc was determined using flame atomic absorption spectrophotometry (PerkinElmer A Analyst 800), in the Department of Human Nutrition, University of Otago. The accuracy and precision of the analyses were checked using controls and in-house pooled samples (after every 15 samples). The analysed mean (SD, CV) value for the zinc control (UTAK Laboratories, Inc., US) was 65.76 μmol/L (1.91, 2.9%), compared to manufacturers’ concentration of 65 μmol/L. Because the samples were collected from infants in the morning, low plasma zinc concentrations were defined as a concentration <9.9 μmol/L (IZiNCG, 2007). For the purpose of this thesis ‘plasma zinc concentration’ is referred to as ‘biochemical zinc status’, in the absence of a more appropriate biomarker for determining biochemical zinc status.

3.11.3 Analysis of inflammatory markers

C-reactive protein (CRP), and α\(_1\)-acid glycoprotein (AGP) were determined using a Cobas C311 automatic electronic analyser (Roche, New Zealand), in the Department of Human Nutrition, University of Otago. The accuracy and precision of the analyses were checked using controls and in-house pooled samples (after every 15 samples). The mean (SD, CV) for the CRP control (Roche Diagnostics, US) was 9.46 mg/L (0.43 mg/L, 4.6%), compared with the manufacturer’s concentration of 9.06 mg/L. The multilevel controls for AGP
(Roche Diagnostics, US) were 0.49 g/L (0.01 g/L, 1.1%) and 0.83 g/L (0.01 g/L, 1.4%), compared with the manufacturer’s concentrations of 0.71 g/L and 1.19 g/L, respectively. Cut offs of >5 mg/L CRP and >1 g/L AGP defined the presence of inflammation, for example as a result of infection (Thurnham et al., 2010). Ferritin multipliers were used to adjust ferritin concentration to remove the influence of inflammation: ferritin*0.88 when CRP >5 mg/L (incubation), ferritin*0.48 when CRP >5 mg/L and AGP >1 g/L (early convalescence), and ferritin*0.70 when AGP >1 g/L (late convalescence) (Thurnham et al., 2010).

3.11.4 Selenium analysis
Selenium concentrations were determined using Inductively Coupled Plasma Mass Spectrometry in the Department of Chemistry, University of Otago. The analysed mean (SD, CV) value for the selenium control (UTAK Laboratories, Inc., US) was 104 ng/mL (1.31, 1.27%), compared to manufacturers’ concentration of 108 ng/mL.

3.12 Adverse events
Participants with biochemical results outside the pre-defined cut offs (plasma ferritin 15-150 μg/L, haemoglobin 105-140 g/L, packed cell volume 0.32-0.41, mean cell volume 70-86 fl, mean cell haemoglobin 23-30 pg) were contacted by letter to inform them of the abnormal result and advised to visit their general practitioner for advice on any necessary action (Appendix R). With permission, the participant’s general practitioner was sent a letter (adapted from a template to include the participant’s name) informing them of the study and the participant’s biochemical results (Appendix R).

3.13 Statistical analysis
The statistical analyses relevant to this thesis are discussed within each chapter because these vary from chapter to chapter.
4 Impact of a baby-led approach to complementary feeding on iron intake and status

6-month-old Quinn consumes iron-fortified infant cereal on a strip of toast
4.1 Introduction

Recommended intakes of iron are often not consumed during the complementary feeding period (World Health Organization, 2004b). It is important that complementary foods high in iron are introduced at 6 months of age in order to maintain adequate iron status (Ministry of Health, 2008). Health professionals have expressed some concern that because foods fed using Baby-Led Weaning (BLW) must be in a form that the infant can pick up and feed themselves, BLW has the potential to increase the risk of iron deficiency if the majority of first foods offered are foods low in iron, such as fruits and vegetables (Brown et al., 2011a). Iron-fortified infant cereal is an important source of iron in this age group (Walter et al., 1993), however, it is not likely to be consumed in sufficient amounts as this semi-liquid food is difficult to self-feed (Morison et al., 2016). Other iron rich foods such as red meat may be offered in a way that can be easily picked up by a 6 month old (Cameron et al., 2013; Szymlek-Gay et al., 2009), but may be avoided due to parental concerns about choking (Cameron et al., 2012b; D’Andrea et al., 2016). A recent observational study reported that mean dietary iron intake in infants following BLW was less than half that of infants following more traditional spoon-feeding (Morison et al., 2016). However, the impact of this lower mean intake on biochemical iron status in infants has not been examined in that (Morison et al., 2016) or any study.

Poor iron intakes can lead to iron deficiency, a common nutritional deficiency globally (Forouzanfar et al., 2016), and iron deficiency that progresses to iron deficiency anaemia can impact on central nervous system growth and development during infancy, leading to poorer cognitive and behavioural performance (Domellöf et al., 2014). Moreover, these impacts on infant development may not be reversible (Lozoff et al., 2006; Congdon et al., 2012). New Zealand data suggest that approximately 20-30% of 6-24 month olds have suboptimal iron status, of whom 4% have iron deficiency anaemia (Soh et al., 2004), and that this is due to insufficient iron intakes at a time when iron requirements are high (Soh et al., 2001). It is important, therefore, to
ensure that baby-led approaches do not increase the risk of iron deficiency before they can be recommended as a suitable alternative to traditional spoon-feeding.

The objective addressed in this chapter was to determine the iron intake (at 7 and 12 months of age) and iron status (at 12 months of age) of infants following BLISS compared with those of infants following traditional spoon-feeding.
4.2 Methods

4.2.1 Data collection

Refer to Chapter 3 for a description of the full methods of data collection. Briefly, this randomised controlled trial included 206 participants allocated to Control or BLISS groups. Both groups received standard midwifery and Well Child care and BLISS participants received eight additional visits (from before birth to 9 months) providing education and support on the BLISS approach to complementary feeding (i.e. Baby-Led Weaning modified to increase iron intake). Intake of iron and its key absorption modifiers was assessed at 7 and 12 months by weighed three-day diet records (WDRs) (Chapter 3, Section 3.8), and a blood sample was collected at 12 months to determine plasma ferritin, haemoglobin, soluble transferrin receptor, C-reactive protein (CRP), and α1-acid glycoprotein (AGP) concentrations; and body iron was calculated (Chapter 3, Section 3.10).

4.2.2 Statistical analysis

The data were analysed according to modified intention to treat and all analyses were conducted using statistical software Stata, Version 14.2 (StataCorp LP, Texas, US). The BLISS study included 206 participants which allowed for a 15% drop-out, with 80% power (α=0.05) to detect a difference in geometric mean plasma ferritin concentration of 5.0 μg/L with 84 participants per group (Daniels et al., 2015). Maternal and infant characteristics of participants who provided intake data at 7 or 12 months of age or blood test data at 12 months of age were compared with those who did not provide data using two-sample t-tests (continuous variables) or chi-squared tests (categorical variables) as appropriate. The proportion of infants at 7 and 12 months of age fed breast milk, infant formula, or breast milk and infant formula, as well as those consuming cow's milk as a drink were determined using Chi-squared tests.

All nutrient and food group data are presented as daily averages of the three days of WDR collection. As the median intakes of some food groups were
very small (e.g., median intake of ‘legumes’ = 0.0 g), these food groups were collapsed with other small food groups of similar composition as follows: 1) ‘legumes’, ‘eggs’, and ‘nuts and seeds’ were combined; 2) ‘beverages’ and ‘miscellaneous’ were combined; 3) ‘cow’s milk’ and ‘dairy’ were combined; and 4) ‘fish and poultry’ and ‘other meat’ were combined. Combination 1 was made as they are all good sources of protein and contain relatively equal amounts of iron (on average 2 mg iron per 100 g), combinations 2 and 3 were made as they all contain little or no iron, and combination 4 was made as both these food groups are considered higher sources of iron than ‘vegetables’, for example, but lower than ‘red meat’.

As most variables were positively skewed, the data were reported as medians and lower and upper quartiles (25th and 75th). Quantile regression was used to estimate the difference between the Control and BLISS groups for energy and nutrient intake, as well as dietary iron intake from each food group.

‘Usual’ iron intake was determined, as three days of dietary data alone does not provide an estimation of ‘usual’ dietary intakes (Harttig et al., 2011). Data from each participant’s WDR was imported into the multiple source method web-based programme (Harttig et al., 2011). The programme calculated the ‘usual’ intake of iron for each participant by accounting for intra-individual variation (Harttig et al., 2011). The prevalence of inadequate intakes of iron at 7 and 12 months was then estimated using the full probability approach. The full probability approach was calculated by categorising each participant’s mean ‘usual’ iron intake into one of 14 specified ranges of intake, defined by the Institute of Medicine (Institute of Medicine, 2002). Following this, the percentage of participants falling within each range was then multiplied by the associated probability of inadequate iron intake for that intake level so that the total of all percentages represented the total probability of inadequate iron intakes at 7 and 12 months (Institute of Medicine, 2002; Gibson et al., 2008). The age ranges used were 8-12 months and 1-3 years for 7 and 12 months, respectively; this was considered appropriate as all WDRs were collected when the respective age had been reached.
Plasma ferritin concentrations were adjusted to account for the effect of inflammation and infection using ferritin multipliers provided by Thurnham et al. (2010): ferritin*0.88 (CRP >5 mg/L), ferritin*0.48 (CRP >5 mg/L, and AGP >1 g/L), and ferritin*0.70 (AGP >1 g/L). A plasma ferritin cut off of <15 μg/L was used to determine depleted iron stores. Although a cut off for plasma ferritin of <12 μg/L is commonly used to define low iron stores (World Health Organization, 2001), when this value was used in initial analyses no participant was classified as having depleted iron stores. It may be that by using body iron and haemoglobin to determine early functional iron deficiency and iron deficiency anaemia, toddlers who would otherwise have been categorised as iron depleted, were being categorised into early functional iron deficiency or iron deficiency anaemia, leading to an overestimation of toddlers with either of these stages of iron deficiency. It was therefore decided to use a cut off of <15 μg/L to define iron depletion in this analysis, as <15 μg/L is the cut off used by the clinical laboratory that carried out the plasma ferritin assays (Southern Community Laboratories Ltd. (SCL)), and values <15 μg/L have previously been used to determine iron deficiency in this age group in some studies (Oti-Boateng et al., 1998; Capozzi et al., 2010).

Means and standard deviations were used to describe all of the biochemical variables except for plasma ferritin, CRP and AGP, which were presented as medians and lower and upper quartiles (25th and 75th), because the data were skewed. Differences in iron status indices were estimated using regression analysis and were adjusted for infant age at the time of blood test, infant sex, maternal education (non-tertiary vs tertiary) and maternal parity (1 child vs >1 child, including the current pregnancy). These adjustment factors were decided a priori. A Chi-squared test was used to compare the number of Control and BLISS infants in each of the iron status categories, and their associated odds ratios.
Table 4.1 Characteristics of participants who provided intake data at 7 (n=162) and/or 12 (n=143) months of age or biochemical data at 12 (n=119) months of age

<table>
<thead>
<tr>
<th>Maternal and household variables</th>
<th>Control (n=81)</th>
<th>BLISS (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at birth, years [mean (SD)]</td>
<td>32.2 (5.8)</td>
<td>31.7 (4.8)</td>
</tr>
</tbody>
</table>

Maternal parity

<table>
<thead>
<tr>
<th></th>
<th>Control (n=81)</th>
<th>BLISS (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First child</td>
<td>32 (40)</td>
<td>37 (42)</td>
</tr>
<tr>
<td>Two children</td>
<td>27 (33)</td>
<td>37 (42)</td>
</tr>
<tr>
<td>Three or more children</td>
<td>22 (27)</td>
<td>14 (16)</td>
</tr>
</tbody>
</table>

Maternal ethnicity

<table>
<thead>
<tr>
<th></th>
<th>Control (n=81)</th>
<th>BLISS (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ European</td>
<td>70 (87)</td>
<td>71 (80)</td>
</tr>
<tr>
<td>Māori</td>
<td>6 (7)</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (6)</td>
<td>9 (10)</td>
</tr>
</tbody>
</table>

Maternal education

<table>
<thead>
<tr>
<th></th>
<th>Control (n=81)</th>
<th>BLISS (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>School only</td>
<td>23 (28)</td>
<td>26 (30)</td>
</tr>
<tr>
<td>Post-secondary</td>
<td>13 (16)</td>
<td>20 (22)</td>
</tr>
<tr>
<td>University</td>
<td>45 (56)</td>
<td>42 (48)</td>
</tr>
</tbody>
</table>

Household deprivation<sup>b</sup>

<table>
<thead>
<tr>
<th></th>
<th>Control (n=81)</th>
<th>BLISS (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 (Low)</td>
<td>24 (30)</td>
<td>25 (28)</td>
</tr>
<tr>
<td>4-6</td>
<td>37 (45)</td>
<td>46 (53)</td>
</tr>
<tr>
<td>7-10 (High)</td>
<td>20 (25)</td>
<td>17 (19)</td>
</tr>
</tbody>
</table>

Infant variables

<table>
<thead>
<tr>
<th></th>
<th>Control (n=81)</th>
<th>BLISS (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>37 (46)</td>
<td>50 (57)</td>
</tr>
<tr>
<td>Male</td>
<td>44 (54)</td>
<td>38 (43)</td>
</tr>
<tr>
<td>Infant birth weight, g [mean (SD)]</td>
<td>3510 (453)</td>
<td>3496 (448)</td>
</tr>
<tr>
<td>Infant gestational age at birth, weeks [mean (SD)]</td>
<td>39.5 (1.2)</td>
<td>39.7 (1.0)</td>
</tr>
</tbody>
</table>

Abbreviations: NZ European, New Zealand European

<sup>a</sup>Data presented as n (%), unless otherwise stated

<sup>b</sup>Household deprivation categorised using the NZDep2013 scale in which decile 1 indicates the lowest level of deprivation and 10 indicates the highest (Atkinson et al., 2014)
4.3 Results

4.3.1 Participant characteristics

A total of 214 mother-infant pairs were randomised, of whom eight were excluded after birth (\(n=5\) Control, \(n=3\) BLISS), providing a final sample size of 206 participants (Chapter 3, Figure 3.1). Of these 206, 169 (82%) provided a WDR (intake data) or a biochemical sample (status data) for analysis. Baseline demographic data are illustrated in Table 4.1 for those providing any infant iron intake or biochemical data. Parent participants were 32 years of age on average when their infant was born, 41% were primiparous, and 83% were New Zealand European. Half (51%) had a university qualification. Overall, 22% had a high level of household deprivation (compared to 30% for the New Zealand population (Atkinson et al., 2014)). Participants who provided data were similar to those who did not for all variables shown, with the exception of maternal age (which was higher for those who provided data; Table 4.2).
Table 4.2 Comparisons of those included (provided either intake or status data) and not included (provided neither intake nor status data) in this data set

<table>
<thead>
<tr>
<th>Maternal and household variables</th>
<th>Included (n=169)</th>
<th>Not included (n=37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at birth, years [mean (SD)]</td>
<td>31.9 (5.3)</td>
<td>28.3 (5.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal parity</td>
<td></td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td>First child</td>
<td>69 (41)</td>
<td>16 (43)</td>
<td></td>
</tr>
<tr>
<td>Two children</td>
<td>64 (38)</td>
<td>11 (30)</td>
<td></td>
</tr>
<tr>
<td>3 or more children</td>
<td>36 (21)</td>
<td>10 (27)</td>
<td></td>
</tr>
<tr>
<td>Maternal ethnicity</td>
<td></td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>NZ European</td>
<td>141 (83)</td>
<td>27 (73)</td>
<td></td>
</tr>
<tr>
<td>Māori</td>
<td>14 (8.5)</td>
<td>6 (16)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>14 (8.5)</td>
<td>4 (11)</td>
<td></td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>School only</td>
<td>49 (29)</td>
<td>14 (38)</td>
<td></td>
</tr>
<tr>
<td>Post-secondary</td>
<td>33 (20)</td>
<td>10 (27)</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>87 (51)</td>
<td>13 (35)</td>
<td></td>
</tr>
<tr>
<td>Household deprivation&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>1-3 (Low)</td>
<td>49 (29)</td>
<td>11 (30)</td>
<td></td>
</tr>
<tr>
<td>4-6</td>
<td>83 (49)</td>
<td>19 (51)</td>
<td></td>
</tr>
<tr>
<td>7-10 (High)</td>
<td>37 (22)</td>
<td>7 (19)</td>
<td></td>
</tr>
</tbody>
</table>
### Infant variables

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>87 (51)</td>
<td>22 (61)</td>
</tr>
<tr>
<td><strong>Infant birth weight, g [mean (SD)]</strong></td>
<td>3503 (449)</td>
<td>3619 (545)</td>
</tr>
<tr>
<td><strong>Infant gestational age at birth, weeks [mean (SD)]</strong></td>
<td>39.6 (1.1)</td>
<td>39.6 (1.0)</td>
</tr>
</tbody>
</table>

Abbreviations: NZ European, New Zealand European

**Bold** indicates a statistically significant difference at $p<0.05$

aData presented as $n$ (%), unless otherwise stated

bHousehold deprivation categorised using the NZDep2013 scale in which decile 1 indicates the lowest level of deprivation and 10 indicates the highest (Atkinson et al., 2014)
4.3.2 Adherence to a baby-led approach and to delaying the introduction of complementary foods

Adherence to the baby-led approach was high with infants in the BLISS group significantly, and substantially, more likely to have fed themselves most or all of their food in the past week than infants in the Control group at 7 (74% vs 19%; \( p < 0.001 \)) and 12 (77% vs 48%; \( p < 0.001 \)) months of age. On average, BLISS infants were introduced to complementary foods later than those in the Control group (mean: 24.6 weeks vs 22.6 weeks, \( p < 0.001 \)) so that the introduction of complementary foods was delayed until 6 months of age (180 days) for 66% of BLISS infants compared to 18% of Control infants (\( p < 0.001 \)).

4.3.3 Infant milk intakes

The range of iron concentrations in the infant formulas consumed were 4-9 mg per 100 g at 7 months of age, and 6-10 mg per 100 g at 12 months of age. There was no difference in the number of infants who were breastfed, formula fed or mixed fed (infant formula and breast milk) between the Control and BLISS groups at either time point (Table 4.3). Only one Control participant consumed cow’s milk at 7 months of age.
Table 4.3 Milk consumers at 7 and 12 months of age\textsuperscript{a,b}

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Control</th>
<th>BLISS</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7 months of age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk only</td>
<td>82 (51)</td>
<td>38 (49)</td>
<td>44 (52)</td>
<td>0.95</td>
</tr>
<tr>
<td>Infant formula only</td>
<td>39 (24)</td>
<td>19 (25)</td>
<td>20 (23)</td>
<td></td>
</tr>
<tr>
<td>Mixed (breast milk and infant formula)</td>
<td>41 (25)</td>
<td>20 (26)</td>
<td>21 (25)</td>
<td></td>
</tr>
<tr>
<td><strong>12 months of age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk only</td>
<td>62 (43)</td>
<td>31 (46)</td>
<td>31 (41)</td>
<td>0.94</td>
</tr>
<tr>
<td>Infant formula only</td>
<td>47 (33)</td>
<td>22 (32)</td>
<td>25 (33)</td>
<td></td>
</tr>
<tr>
<td>Mixed (breast milk and infant formula)</td>
<td>15 (11)</td>
<td>7 (10)</td>
<td>8 (11)</td>
<td></td>
</tr>
<tr>
<td>None of the above</td>
<td>19 (13)</td>
<td>8 (12)</td>
<td>11 (15)</td>
<td></td>
</tr>
<tr>
<td><strong>Cow’s milk</strong>\textsuperscript{c}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>92 (64)</td>
<td>47 (69)</td>
<td>45 (60)</td>
<td>0.51</td>
</tr>
<tr>
<td>&lt;500 mL/day</td>
<td>40 (28)</td>
<td>17 (25)</td>
<td>23 (31)</td>
<td></td>
</tr>
<tr>
<td>(\geq 500) mL/day</td>
<td>11 (8)</td>
<td>4 (6)</td>
<td>7 (9)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data presented as \(n\) (%)

\textsuperscript{b}Based on intake reported during the three-day weighed diet records, collected at 7 and 12 months

\textsuperscript{c}Cow’s milk consumed as a drink
4.3.4 Intakes of iron and iron absorption modifiers

There were no statistically significant differences in dietary iron intake between the groups at either 7 (difference 0.6 mg/day; 95% CI: -1.0, 2.3; p=0.46) or 12 (difference -0.1 mg/day, 95% CI: -1.6, 1.4, p=0.87) months of age. Similarly, no statistically significant group differences in the intakes of haem iron, non-haem iron, ‘meat, fish, poultry’, phytate, or phytate-to-iron molar ratio were observed at either age (Tables 4.4 and 4.5).

The only significant difference observed in the intake of an iron absorption modifier was a significantly lower intake of Vitamin C in BLISS infants (49.2 mg/day) compared with Control infants (59.2 mg/day; difference -9.7 mg; 95% CI: -18.4, -0.9; p=0.032). However, this was only at 7 months of age, and there were no infants with a Vitamin C intake less than the EAR (25 mg/day (Department of Health and Ageing Australia et al., 2006)) in either group. Only two participants consumed tea as part of their diets (n=1 Control participant at 7 months, n=1 Control participant at 12 months).

Two participants in each group were taking iron supplements during the 12 month WDR collection period, as a result of identification of iron deficiency from the blood test that was conducted before the WDR was administered. Because the supplements were not being taken at the time of the blood test, they are not included in the iron intake data reported here.
Table 4.4 Intake of iron and key iron absorption modifiers at 7 months of age from complementary foods and infant milks\(^a,\!^b\)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BLISS</th>
<th>Difference (95% CI)(^c)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 months of age</td>
<td>(n=77)</td>
<td>(n=85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kJ/day [mean (SD)]</td>
<td>2862 (548)</td>
<td>2996 (613)</td>
<td>145 (-31.2, 321)</td>
<td>0.11</td>
</tr>
<tr>
<td>Energy from complementary foods only, kJ/day [mean (SD)](^d)</td>
<td>672 (506)</td>
<td>799 (595)</td>
<td>144 (-26.2, 314)</td>
<td>0.10</td>
</tr>
<tr>
<td>Dietary iron, mg/day</td>
<td>2.7 (1.3, 6.9)</td>
<td>3.0 (1.5, 7.3)</td>
<td>0.6 (-1.0, 2.3)</td>
<td>0.46</td>
</tr>
<tr>
<td>Dietary iron from complementary foods only, mg/day(^e)</td>
<td>1.0 (0.5, 2.2)</td>
<td>1.2 (0.7, 2.0)</td>
<td>0.2 (-0.2, 0.6)</td>
<td>0.34</td>
</tr>
<tr>
<td>Haem iron, mg/day</td>
<td>0.0 (0.0, 0.1)</td>
<td>0.0 (0.0, 0.1)</td>
<td>0.0 (-0.0, 0.1)</td>
<td>0.10</td>
</tr>
<tr>
<td>Non-haem iron, mg/day</td>
<td>2.6 (1.3, 6.9)</td>
<td>2.9 (1.4, 7.3)</td>
<td>0.4 (-1.3, 2.0)</td>
<td>0.67</td>
</tr>
<tr>
<td>Meat, fish, poultry, g/day</td>
<td>2.8 (0.0, 11.1)</td>
<td>4.3 (1.4, 8.8)</td>
<td>1.3 (-1.9, 4.4)</td>
<td>0.42</td>
</tr>
<tr>
<td>Phytate, mg/day</td>
<td>36 (16.3, 75.2)</td>
<td>45 (23.0, 77.6)</td>
<td>4.2 (-15.0, 23.4)</td>
<td>0.67</td>
</tr>
<tr>
<td>Phytate:iron molar ratio(^f)</td>
<td>1.0 (0.4, 2.3)</td>
<td>1.3 (0.6, 2.7)</td>
<td>0.4 (-0.2, 1.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Vitamin C, mg/day</td>
<td>59.2 (41.7, 75.6)</td>
<td>49.2 (38.3, 67.9)</td>
<td>-9.7 (-18.4, -0.9)</td>
<td>0.032</td>
</tr>
<tr>
<td>Prescribed iron supplements [(n) (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(\text{Bold}\) indicates a statistically significant difference at \(p<0.05\)

\(^a\)Data presented as median (25\(^{th}\), 75\(^{th}\) percentile), unless otherwise stated
\(^b\)Intake reported during the three-day weighed diet records collected at 7 months
\(^c\)Difference adjusted for infant age and sex, and maternal education and parity
\(^d\)Excludes intake from breast milk and infant formula
\(^e\)Excludes iron from breast milk and infant formula
\(^f\)Calculated as \([\text{phytate (mg)} / 660] / [\text{iron (mg)} / 55.9]\)
Table 4.5 Intake of iron and key iron absorption modifiers at 12 months of age from complementary foods and infant milks\textsuperscript{a,b}

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BLISS</th>
<th>Difference (95% CI)\textsuperscript{c}</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>12 months of age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kJ/day [mean (SD)]</td>
<td>3573 (776)</td>
<td>3623 (1048)</td>
<td>109 (-191, 409)</td>
<td>0.48</td>
</tr>
<tr>
<td>Energy from complementary foods only, kJ/day [mean (SD)]\textsuperscript{d}</td>
<td>2400 (848)</td>
<td>2527 (1183)</td>
<td>195 (-142, 533)</td>
<td>0.25</td>
</tr>
<tr>
<td>Dietary iron, mg/day</td>
<td>5.3 (3.1, 8.4)</td>
<td>4.7 (3.1, 7.3)</td>
<td>-0.1 (-1.6, 1.4)</td>
<td>0.87</td>
</tr>
<tr>
<td>Dietary iron from complementary foods only, mg/day\textsuperscript{e}</td>
<td>3.2 (2.3, 4.6)</td>
<td>3.2 (2.5, 4.1)</td>
<td>-0.0 (-0.6, 0.6)</td>
<td>0.94</td>
</tr>
<tr>
<td>Haem iron, mg/day</td>
<td>0.2 (0.1, 0.3)</td>
<td>0.2 (0.1, 0.3)</td>
<td>0.0 (-0.0, 0.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Non-haem iron, mg/day</td>
<td>5.0 (2.9, 8.1)</td>
<td>4.5 (2.9, 7.0)</td>
<td>-0.1 (-1.7, 1.4)</td>
<td>0.85</td>
</tr>
<tr>
<td>Meat, fish, poultry, g/day</td>
<td>19.3 (7.9, 33.6)</td>
<td>19.3 (11.2, 31.1)</td>
<td>-1.4 (-9.0, 6.2)</td>
<td>0.72</td>
</tr>
<tr>
<td>Phytate, mg/day</td>
<td>187 (118, 310)</td>
<td>229 (152, 274)</td>
<td>37 (-20.4, 94.8)</td>
<td>0.20</td>
</tr>
<tr>
<td>Phytate:iron molar ratio\textsuperscript{f}</td>
<td>3.8 (2.3, 6.2)</td>
<td>4.3 (2.8, 6.5)</td>
<td>0.6 (-0.7, 1.9)</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin C, mg/day</td>
<td>48.1 (39.4, 69.5)</td>
<td>50.4 (36.6, 61.4)</td>
<td>0.4 (-9.4, 10.3)</td>
<td>0.93</td>
</tr>
<tr>
<td>Prescribed iron supplements [n (%)]</td>
<td>2 (3.4)\textsuperscript{g}</td>
<td>2 (3.3)\textsuperscript{g}</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data presented as median (25\textsuperscript{th}, 75\textsuperscript{th} percentile), unless otherwise stated
\textsuperscript{b}Intake reported during the three-day weighed diet records collected at 12 months
\textsuperscript{c}Difference adjusted for infant age and sex, and maternal education and parity
\textsuperscript{d}Excludes intake from breast milk and infant formula
\textsuperscript{e}Excludes iron from breast milk and infant formula
\textsuperscript{f}Calculated as [phytate (mg) / 660] / [iron (mg) / 55.9]
\textsuperscript{g}All four participants who reported taking iron supplements in the 12 month WDR started doing so after the 12 month blood sample had been collected
4.3.5 Infant milk intakes and their contribution to iron intakes

There were no significant differences in the estimated intake of breast milk or measured intake of infant formula between BLISS and Control infants at 7 (breast milk difference: 0.0 g/day; 95% CI: -5.1, 5.1; p=1.00; infant formula difference: 216 g/day; 95% CI: -97.2, 530; p=0.17) or 12 (breast milk difference: -0.0 g/day; -0.1, 0.1; p=0.94; infant formula difference: -84.8 g/day; -277, 107; p=0.38) months. Correspondingly, there were no differences between groups in the contribution of infant milks to dietary iron intakes (all p>0.17).

4.3.6 Contribution of complementary foods to iron intakes

Tables 4.6 and 4.7 illustrate the contribution of different complementary foods to iron intake at 7 and 12 months, respectively. Although no significant difference was observed in overall iron intakes, the amount of iron from each food group differed according to intervention group. BLISS infants obtained significantly more iron from ‘breads and cereals’ (not including ‘iron-fortified infant cereal’, p<0.001), ‘red meat’ (p=0.01), ‘dairy’ (p=0.01), and ‘legumes, nuts, seeds and eggs’ (p=0.001) than Control infants at 7 months of age (Table 4.6). In the case of all these food groups, except ‘breads and cereals’, this reflected the greater proportion of BLISS infants consuming these foods (Table 4.8).

However, the actual differences in iron contribution were small (differences of 0.0 to 0.2 mg/day) in comparison to the EAR of 4.0 mg/day (Department of Health and Ageing Australia et al., 2006) and therefore not likely to be of clinical significance. For example, the BLISS group obtained more iron from ‘red meat’ than the Control group but the median iron intakes were 0.06 mg/day in BLISS infants and 0.01 mg/day in Control infants, representing just 5% (BLISS) and 1% (Control) of total dietary iron from complementary foods.

None of the differences apparent at 7 months remained at 12 months, and although BLISS infants did receive significantly less iron from ‘vegetables’ than Control infants, the actual difference was small (difference -0.1 mg/day; 95% CI: -0.2, -0.0; p=0.027) (Table 4.7). Also, this small difference did not reflect
a significantly different number of consumers of ‘vegetables’ between groups at 12 months (Table 4.8).
**Table 4.6 Iron from complementary foods at 7 months of age (consumers and non-consumers)\textsuperscript{a,b,c}**

<table>
<thead>
<tr>
<th></th>
<th>Control mg/day</th>
<th>%\textsuperscript{d}</th>
<th>BLISS mg/day</th>
<th>%\textsuperscript{d}</th>
<th>Difference (95% CI)\textsuperscript{e}</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7 months of age</strong></td>
<td>n=77</td>
<td>n=85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td>0.16 (0.0, 0.4)</td>
<td>17 (9, 25)</td>
<td>0.10 (0.0, 0.2)</td>
<td>8.4 (6, 17)</td>
<td>-0.1 (-0.1, 0.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>0.13 (0.0, 0.2)</td>
<td>11 (5, 24)</td>
<td>0.09 (0.0, 0.2)</td>
<td>7.2 (3, 12)</td>
<td>-0.0 (-0.1, 0.0)</td>
<td>0.19</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>0.08 (0.0, 0.7)</td>
<td>7.9 (0, 54)</td>
<td>0.19 (0.0, 0.5)</td>
<td>19 (0, 43)</td>
<td>0.1 (-0.1, 0.3)</td>
<td>0.25</td>
</tr>
<tr>
<td>Breads and cereals\textsuperscript{f}</td>
<td>0.09 (0.0, 0.3)</td>
<td>7.2 (2, 26)</td>
<td>0.26 (0.1, 0.4)</td>
<td>23 (10, 35)</td>
<td>0.2 (0.1, 0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Red meat\textsuperscript{g}</td>
<td>0.01 (0.0, 0.2)</td>
<td>1.9 (0, 14)</td>
<td>0.06 (0.0, 0.2)</td>
<td>7.2 (1, 16)</td>
<td>0.1 (0.0, 0.1)</td>
<td>0.010</td>
</tr>
<tr>
<td>Miscellaneous\textsuperscript{h}</td>
<td>0.01 (0.0, 0.1)</td>
<td>1.1 (0, 6)</td>
<td>0.01 (0.0, 0.1)</td>
<td>1.3 (0, 6)</td>
<td>0.0 (-0.0, 0.0)</td>
<td>0.75</td>
</tr>
<tr>
<td>Dairy</td>
<td>0.00 (0.0, 0.0)\textsuperscript{i}</td>
<td>0.1 (0, 0.4)</td>
<td>0.00 (0.0, 0.0)</td>
<td>0.5 (0, 2)</td>
<td>0.0 (0.0, 0.0)</td>
<td>0.010</td>
</tr>
<tr>
<td>Legumes, nuts, seeds and eggs</td>
<td>0.00 (0.0, 0.0)</td>
<td>0.0 (0, 2)</td>
<td>0.04 (0.0, 0.1)</td>
<td>4.5 (1, 11)</td>
<td>0.0 (0.0, 0.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Other meat\textsuperscript{j}</td>
<td>0.00 (0.0, 0.0)</td>
<td>0.0 (0, 3)</td>
<td>0.00 (0.0, 0.0)</td>
<td>0.4 (0, 4)</td>
<td>0.0 (-0.0, 0.0)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

**Bold** indicates a statistically significant difference at p<0.05
\textsuperscript{a}Data presented as median (25\textsuperscript{th}, 75\textsuperscript{th} percentile)
\textsuperscript{b}Intake reported during the three-day weighed diet records collected at 7 months
\textsuperscript{c}Ordered from highest to lowest contributor of iron to the intakes of the Control group
\textsuperscript{d}Data expressed as median percentages (NB: mean percentages added to 100% of total iron intakes from complementary foods)
\textsuperscript{e}Difference in median iron (mg/day) intake between groups: negative values represent lower values in BLISS than in Control, positive values represent higher values in BLISS than in Control, adjusted for infant age and sex, and maternal education and parity
\textsuperscript{f}Breads and cereals other than iron-fortified infant cereals
\textsuperscript{g}Red meat defined as: beef, lamb, mutton, venison
\textsuperscript{h}Miscellaneous defined as: fats, sugar, sweet foods, herbs and spices, sauces, spreads, beverages etc.
\textsuperscript{i}Where the median intake is 0.00 this has occurred because more than half of the infants did not consume this food. Some infants did consume these foods, however, so it was possible for differences in intake to be significant. Similarly, the difference will be reported as 0.00 if it is smaller than 0.05 and therefore rounds down to 0.00.
\textsuperscript{j}Other meat defined as: fish, poultry, pork, processed meats
Table 4.7 Iron from complementary foods at 12 months of age (consumers and non-consumers)\textsuperscript{ab,c}

<table>
<thead>
<tr>
<th>Food Category</th>
<th>Control mg/day</th>
<th>Control %d</th>
<th>BLISS mg/day</th>
<th>BLISS %d</th>
<th>Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 months of age</td>
<td>n=68</td>
<td>n=75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breads and cereals\textsuperscript{1}</td>
<td>0.84 (0.5, 1.6)</td>
<td>32 (16, 48)</td>
<td>1.10 (0.6, 1.8)</td>
<td>38 (27, 50)</td>
<td>0.2 (-0.2, 0.5)</td>
<td>0.26</td>
</tr>
<tr>
<td>Vegetables</td>
<td>0.38 (0.2, 0.5)</td>
<td>11 (6, 16)</td>
<td>0.29 (0.1, 0.5)</td>
<td>8.9 (4, 14)</td>
<td>-0.1 (-0.2, -0.0)</td>
<td>0.027</td>
</tr>
<tr>
<td>Miscellaneous\textsuperscript{6}</td>
<td>0.32 (0.1, 0.6)</td>
<td>9.8 (4, 18)</td>
<td>0.18 (0.1, 0.5)</td>
<td>5.7 (2, 17)</td>
<td>-0.1 (-0.3, 0.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>0.27 (0.2, 0.5)</td>
<td>8.3 (5, 13)</td>
<td>0.32 (0.2, 0.5)</td>
<td>10 (5, 14)</td>
<td>0.0 (-0.1, 0.1)</td>
<td>0.33</td>
</tr>
<tr>
<td>Other meat\textsuperscript{h}</td>
<td>0.17 (0.1, 0.3)</td>
<td>5.5 (2, 9)</td>
<td>0.17 (0.1, 0.3)</td>
<td>5.1 (1, 4)</td>
<td>-0.0 (-0.1, 0.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Legumes, nuts, seeds and eggs</td>
<td>0.10 (0.0, 0.3)</td>
<td>2.8 (0, 10)</td>
<td>0.16 (0.0, 0.4)</td>
<td>4.6 (1, 10)</td>
<td>0.0 (-0.0, 0.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Red meat\textsuperscript{i}</td>
<td>0.09 (0.0, 0.3)</td>
<td>2.5 (0, 11)</td>
<td>0.15 (0.0, 0.4)</td>
<td>3.8 (0, 12)</td>
<td>0.1 (-0.1, 0.2)</td>
<td>0.40</td>
</tr>
<tr>
<td>Dairy</td>
<td>0.06 (0.0, 0.1)</td>
<td>1.5 (1, 4)</td>
<td>0.05 (0.0, 0.1)</td>
<td>1.7 (0, 4)</td>
<td>-0.0 (-0.0, 0.0)</td>
<td>0.81</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>0.00\textsuperscript{j}</td>
<td>0.0 (0, 0)</td>
<td>0.00 (0.0, 0.1)</td>
<td>0.0 (0, 5)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textbf{Bold} indicates a statistically significant difference at p<0.05

\textsuperscript{a}Data presented as median (25\textsuperscript{th}, 75\textsuperscript{th} percentile)

\textsuperscript{b}Intake reported during the three-day weighed diet records collected at 12 months

\textsuperscript{c}Ordered from highest to lowest contributor of iron to the intakes of the Control group

\textsuperscript{d}Data expressed as median percentages (NB: mean percentages added to 100% of total iron intakes from complementary foods)

\textsuperscript{e}Difference in median iron (mg/day) intake between groups: negative values represent lower values in BLISS than in Control, positive values represent higher values in BLISS than in Control, adjusted for infant age and sex, and maternal education and parity

\textsuperscript{f}Breads and cereals other than iron-fortified infant cereals

\textsuperscript{g}Miscellaneous defined as: fats, sugar, sweet foods, herbs and spices, sauces, spreads, beverages etc.

\textsuperscript{h}Other meat defined as: fish, poultry, pork, processed meats

\textsuperscript{i}Red meat defined as: beef, lamb, mutton, venison

\textsuperscript{j}Where the median intake is 0.00 this has occurred because more than half of the infants did not consume this food. Some infants did consume these foods, however, so it was possible for differences in intake to be significant. Similarly, the difference will be reported as 0.00 if it is smaller than 0.05 (and therefore rounds down to 0.00).
Table 4.8 Number of consumers of each food group at 7 and 12 months of age\textsuperscript{a,b,c}

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Control</th>
<th>BLISS</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7 months of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breads and cereals\textsuperscript{d}</td>
<td>77 (100)</td>
<td>85 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Miscellaneous\textsuperscript{e}</td>
<td>77 (100)</td>
<td>85 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Vegetables</td>
<td>75 (97)</td>
<td>84 (99)</td>
<td>0.50</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>73 (95)</td>
<td>81 (95)</td>
<td>0.89</td>
</tr>
<tr>
<td>Dairy</td>
<td>66 (86)</td>
<td>82 (96)</td>
<td>0.015</td>
</tr>
<tr>
<td>Breast milk</td>
<td>58 (75)</td>
<td>65 (76)</td>
<td>0.87</td>
</tr>
<tr>
<td>Red meat\textsuperscript{f}</td>
<td>42 (55)</td>
<td>65 (76)</td>
<td>0.003</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>39 (51)</td>
<td>62 (73)</td>
<td>0.003</td>
</tr>
<tr>
<td>Infant formula</td>
<td>39 (51)</td>
<td>41 (48)</td>
<td>0.76</td>
</tr>
<tr>
<td>Other meat\textsuperscript{g}</td>
<td>38 (49)</td>
<td>45 (53)</td>
<td>0.65</td>
</tr>
<tr>
<td>Legumes, nuts, seeds and eggs</td>
<td>26 (34)</td>
<td>71 (84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>12 months of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breads and cereals\textsuperscript{d}</td>
<td>68 (100)</td>
<td>75 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Miscellaneous\textsuperscript{e}</td>
<td>68 (100)</td>
<td>75 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Dairy</td>
<td>68 (100)</td>
<td>74 (99)</td>
<td>0.34</td>
</tr>
<tr>
<td>Vegetables</td>
<td>67 (99)</td>
<td>75 (100)</td>
<td>0.29</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>66 (97)</td>
<td>72 (96)</td>
<td>0.73</td>
</tr>
<tr>
<td>Other meat\textsuperscript{f}</td>
<td>57 (84)</td>
<td>67 (89)</td>
<td>0.33</td>
</tr>
<tr>
<td>Legumes, nuts, seeds and eggs</td>
<td>55 (81)</td>
<td>66 (88)</td>
<td>0.24</td>
</tr>
<tr>
<td>Red meat\textsuperscript{g}</td>
<td>41 (60)</td>
<td>53 (71)</td>
<td>0.19</td>
</tr>
<tr>
<td>Breast milk</td>
<td>38 (56)</td>
<td>39 (52)</td>
<td>0.64</td>
</tr>
<tr>
<td>Infant formula</td>
<td>29 (43)</td>
<td>33 (44)</td>
<td>0.87</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>14 (21)</td>
<td>21 (28)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

\textbf{Bold} indicates a statistically significant difference at \( p < 0.05 \)
\textsuperscript{a}Data presented as \( n \) (%)
\textsuperscript{b}Intake (any) reported during the three-day weighed diet records collected at 7 and 12 months
\textsuperscript{c}Ordered by number of consumers in the Control group from highest to lowest
\textsuperscript{d}Breads and cereals other than iron-fortified infant cereals
\textsuperscript{e}Miscellaneous includes: fats, sugar, sweet foods, herbs and spices, sauces, spreads, beverages etc.
\textsuperscript{f}Red meat defined as: beef, lamb, mutton, venison
\textsuperscript{g}Other meat defined as: fish, poultry, pork, processed meats
4.3.7 Contribution of intervention foods (red meat and iron-fortified infant cereal) to iron intakes

The BLISS intervention specifically encouraged the consumption of ‘red meat’ and ‘iron-fortified infant cereal’ from the start of the complementary feeding period because of their high iron content or bioavailability.

BLISS infants were introduced to ‘red meat’ at the same age as Control infants (28.1 weeks vs 27.9 weeks, \( p=0.74 \)). While more BLISS infants consumed ‘red meat’ at 7 months than Control infants (76% vs 55%, \( p=0.003 \); Table 4.8), median intakes in consumers were similarly low in both groups (BLISS: 3.2 g/day vs Control: 3.8 g/day, \( p=0.36 \); Table 4.8). By contrast, BLISS infants first received ‘iron-fortified infant cereal’ approximately two weeks later than Control infants (25.4 weeks vs 23.7 weeks, \( p=0.008 \)). As was found with ‘red meat’, more BLISS infants were consuming ‘iron-fortified infant cereal’ by 7 months (73% vs 51%, \( p=0.003 \) ) (Table 4.8), but the median amounts eaten by consumers were very small (BLISS: 1.7 g/day vs Control: 4.0 g/day, \( p=0.041 \) ) (Table 4.9).

At 12 months of age there were no significant differences in the number of consumers of ‘iron-fortified infant cereal’ or ‘red meat’ (Table 4.8), or in the amount consumed (Table 4.10)
| Source of Iron | Control | BLISS | Difference
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/day</td>
<td>mg/day</td>
<td>g/day</td>
<td>mg/day</td>
</tr>
<tr>
<td>Infant formula</td>
<td>309 (110, 745)</td>
<td>5.5 (1.2, 8.3)</td>
<td>525 (136, 804)</td>
<td>6.0 (2.7, 7.5)</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>4.0 (2, 9)</td>
<td>0.72 (0.3, 1.3)</td>
<td>1.7 (0.5, 5)</td>
<td>0.37 (0.1, 0.9)</td>
</tr>
<tr>
<td>Breast milk</td>
<td>750 (660, 750)</td>
<td>0.52 (0.46, 0.53)</td>
<td>750 (660, 750)</td>
<td>0.52 (0.48, 0.53)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>34.8 (12, 72)</td>
<td>0.16 (0.1, 0.4)</td>
<td>20.5 (10, 43)</td>
<td>0.10 (0.0, 0.2)</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>55.6 (19, 94)</td>
<td>0.14 (0.1, 0.3)</td>
<td>39.5 (16, 69)</td>
<td>0.10 (0.0, 0.2)</td>
</tr>
<tr>
<td>Red meat f</td>
<td>3.8 (1, 9)</td>
<td>0.13 (0.0, 0.4)</td>
<td>3.2 (1, 6)</td>
<td>0.11 (0.0, 0.2)</td>
</tr>
<tr>
<td>Breads and cereals g</td>
<td>7.8 (2, 18)</td>
<td>0.11 (0.0, 0.3)</td>
<td>15.5 (8, 28)</td>
<td>0.26 (0.1, 0.4)</td>
</tr>
<tr>
<td>Legumes, nuts, seeds and eggs</td>
<td>3.7 (1, 7)</td>
<td>0.06 (0.01, 0.2)</td>
<td>3.1 (1, 9)</td>
<td>0.05 (0.0, 0.2)</td>
</tr>
<tr>
<td>Other meat h</td>
<td>3.6 (2, 8)</td>
<td>0.04 (0.01, 0.1)</td>
<td>4.7 (2, 9)</td>
<td>0.04 (0.02, 0.1)</td>
</tr>
<tr>
<td>Miscellaneous i</td>
<td>40.0 (10, 85)</td>
<td>0.01 (0.0, 0.1)</td>
<td>32.8 (10, 61)</td>
<td>0.02 (0.0, 0.1)</td>
</tr>
<tr>
<td>Dairy</td>
<td>10.8 (0.4, 29)</td>
<td>0.0 (0.0, 0.0)</td>
<td>9.4 (2, 24)</td>
<td>0.0 (0.0, 0.0)</td>
</tr>
</tbody>
</table>

**Bold** indicates a statistically significant difference at $p<0.05$.

aRefer to Table 4.8 for the number of consumers of each food group at 7 months.

bData presented as median (25th, 75th percentile).

cIntake reported during the three-day weighed diet records collected at 7 months.

dOrdered from highest to lowest food group contributing to total iron intakes in the Control group.

eDifference in median iron (mg/day) intake between groups: negative values represent lower values in BLISS than in Control, positive values represent higher values in BLISS than in Control.

fRed meat defined as: beef, lamb, mutton, venison.

gBreads and cereals other than iron-fortified infant cereals.

hOther meat defined as: fish, poultry, pork and processed meats.

iMiscellaneous includes: fats, sugar, sweet foods, herbs and spices, sauces, spreads, beverages etc.
Table 4.10 Dietary sources of iron for consumers only\(^a\) at 12 months of age (complementary foods and infant milks)\(^b,c,d\)

| Food Group                                  | Control \(
g/day\) | Control \(mg/day\) | BLISS \(g/day\) | BLISS \(mg/day\) | Difference \((95\% CI)^e\) | \(p\)  |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant formula</td>
<td>414 (274, 569)</td>
<td>4.9 (3.5, 6.4)</td>
<td>329 (87, 524)</td>
<td>3.8 (1.5, 5.4)</td>
<td>-1.1 (-2.9, 0.7)</td>
<td>0.23</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>7.2 (3, 15)</td>
<td>1.2 (0.6, 3.5)</td>
<td>3.3 (2, 5)</td>
<td>0.73 (0.4, 1.2)</td>
<td>-0.7 (-1.8, 0.4)</td>
<td>0.22</td>
</tr>
<tr>
<td>Breads and cereals(^f)</td>
<td>57.1 (39, 74)</td>
<td>0.84 (0.5, 1.6)</td>
<td>60.2 (47, 82)</td>
<td>1.10 (0.6, 1.8)</td>
<td>0.2 (-0.2, 0.5)</td>
<td>0.26</td>
</tr>
<tr>
<td>Vegetables</td>
<td>64.6 (45, 97)</td>
<td>0.39 (0.2, 0.5)</td>
<td>55.5 (26, 73)</td>
<td>0.29 (0.1, 0.5)</td>
<td>-0.1 (-0.2, -0.0)</td>
<td>0.023</td>
</tr>
<tr>
<td>Miscellaneous(^g)</td>
<td>132 (89, 205)</td>
<td>0.32 (0.1, 0.6)</td>
<td>119 (67, 235)</td>
<td>0.18 (0.1, 0.5)</td>
<td>-0.1 (-0.3, 0.0)</td>
<td>0.053</td>
</tr>
<tr>
<td>Breast milk</td>
<td>448 (448, 448)</td>
<td>0.31 (0.3, 0.31)</td>
<td>448 (443, 448)</td>
<td>0.31 (0.3, 0.31)</td>
<td>-0.0 (-0.0, 0.0)</td>
<td>0.54</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>94.4 (52, 132)</td>
<td>0.27 (0.2, 0.5)</td>
<td>106 (60, 165)</td>
<td>0.32 (0.2, 0.5)</td>
<td>0.1 (-0.0, 0.2)</td>
<td>0.31</td>
</tr>
<tr>
<td>Red meat(^h)</td>
<td>9.2 (5, 19)</td>
<td>0.27 (0.1, 0.6)</td>
<td>9.4 (4, 15)</td>
<td>0.28 (0.1, 0.5)</td>
<td>0.0 (-0.2, 0.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>Other meat(^i)</td>
<td>17.7 (8, 28)</td>
<td>0.21 (0.1, 0.3)</td>
<td>15.7 (8, 27)</td>
<td>0.19 (0.1, 0.3)</td>
<td>-0.0 (-0.1, 0.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>Legumes, nuts, seeds and eggs</td>
<td>7.2 (3, 25)</td>
<td>0.14 (0.0, 0.4)</td>
<td>11.2 (5, 23)</td>
<td>0.20 (0.1, 0.4)</td>
<td>0.1 (-0.0, 0.2)</td>
<td>0.27</td>
</tr>
<tr>
<td>Dairy</td>
<td>84.4 (34, 188)</td>
<td>0.06 (0.0, 0.1)</td>
<td>109 (51, 188)</td>
<td>0.06 (0.0, 0.1)</td>
<td>0.0 (-0.0, 0.0)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

**Bold** indicates a statistically significant difference at \(p<0.05\)

\(^a\)Refer to Table 4.8 for the number of consumers of each food group at 12 months

\(^b\)Data presented as median (25\(^{th}\), 75\(^{th}\) percentile)

\(^c\)Intake reported during the three-day weighed diet records collected at 12 months

\(^d\)Ordered from highest to lowest food group contributing to total iron intakes in the Control group

\(^e\)Difference in median iron (mg/day) intake between groups: negative values represent lower values in BLISS than in Control, positive values represent higher values in BLISS than in Control

\(^f\)Breads and cereals other than iron-fortified infant cereals

\(^g\)Miscellaneous includes: fats, sugar, sweet foods, herbs and spices, sauces, spreads, beverages etc.

\(^h\)Red meat defined as: beef, lamb, mutton, venison

\(^i\)Other meat defined as: fish, poultry, pork, processed meats
4.3.8 Prevalence of inadequate iron intakes
At 7 months, 74% of infants in both groups had inadequate iron intakes. However, the prevalence was considerably lower by 12 months at 23% of Controls and 26% of BLISS infants. By contrast, no participant from either the BLISS or Control groups had total dietary iron intakes above the upper level of intake of 20 mg/day (Department of Health and Ageing Australia et al., 2006).

4.3.9 Biochemical iron status
There were no statistically significant differences between the groups in any of the biochemical indicators of iron status (all \( p > 0.55 \)) (Table 4.11). Because the blood collection was delayed until infants were well, very few participants had any signs of inflammation/infection (\( n = 0 \) with raised CRP alone; \( n = 2 \) Control and \( n = 0 \) BLISS with raised CRP and raised AGP; \( n = 6 \) Control and \( n = 11 \) BLISS with raised AGP alone). The majority of infants in both groups were iron sufficient (83% Control, 83% BLISS), with just 5% of Control (\( n = 3 \)) and 7% of BLISS (\( n = 4 \)) infants presenting with iron deficiency anaemia (OR for Control relative to BLISS: 0.8; 95% CI: 0.16, 3.60; \( p = 0.74 \)) (Table 4.11). Seventeen percent of BLISS and Control infants had suboptimal iron status (i.e. iron depletion, early functional iron deficiency or iron deficiency anaemia). Similar numbers of infants in both groups who were classified as iron sufficient had a type of anaemia other than iron deficiency anaemia, defined as a haemoglobin concentration <110 g/L, body iron ≥0 mg/kg, and plasma ferritin ≥15 μg/L (13% BLISS vs 10% Control, \( p = 0.78 \)).

Thirty-four participants had at least one biochemical value from the complete blood count (ferritin, haemoglobin, hematocrit, mean cell volume, and/or mean cell haemoglobin) outside the expected reference range for their age and were advised to contact their general practitioner for follow up (\( n = 19 \) Control; \( n = 15 \) BLISS).
Table 4.11 Iron status indicators and categories at 12 months of age\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Control ((n=59))</th>
<th>BLISS ((n=60))</th>
<th>Difference (95% CI)(^b)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin, g/L [mean (SD)]</td>
<td>117 (8.4)</td>
<td>116 (8.9)</td>
<td>-0.8 (-4.0, 2.3)</td>
<td>0.59</td>
</tr>
<tr>
<td>Plasma ferritin, (\mu g/L)</td>
<td>28.9 (18.5, 47.4)</td>
<td>27.0 (19.5, 42.1)</td>
<td>-2.6 (-10.9, 5.8)</td>
<td>0.55</td>
</tr>
<tr>
<td>Soluble transferrin receptor, mg/L [mean (SD)]</td>
<td>7.6 (2.0)</td>
<td>7.4 (2.7)</td>
<td>-0.2 (-1.0, 0.7)</td>
<td>0.70</td>
</tr>
<tr>
<td>Body iron, mg/kg [mean (SD)](^d)</td>
<td>3.3 (3.1)</td>
<td>3.3 (2.9)</td>
<td>0.04 (-1.1, 1.2)</td>
<td>0.95</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>0.1 (0.0, 0.5)</td>
<td>0.2 (0.1, 0.5)</td>
<td>-0.02 (-0.2, 0.2)</td>
<td>0.86</td>
</tr>
<tr>
<td>(\alpha_1)-acid glycoprotein, g/L</td>
<td>0.6 (0.4, 0.8)</td>
<td>0.6 (0.5, 0.95)</td>
<td>0.04 (-0.1, 0.2)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

**Iron status categories, \(n\) (%)**

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)(^e)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron sufficient(^f)</td>
<td>1.0 (reference)</td>
<td>-</td>
</tr>
<tr>
<td>Iron depleted(^g)</td>
<td>1.5 (0.2, 9.6)</td>
<td>0.65</td>
</tr>
<tr>
<td>Early functional iron deficiency(^h)</td>
<td>1.0 (0.2, 4.3)</td>
<td>0.98</td>
</tr>
<tr>
<td>Iron deficiency anaemia(^i)</td>
<td>0.8 (0.2, 3.6)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\(^a\) Data presented as median (25th, 75th percentile), unless otherwise stated
\(^b\) Difference adjusted for infant age and sex, and maternal education and parity: negative values represent lower values in BLISS than in Control, positive values represent higher values in BLISS than in Control
\(^c\) Ferritin adjusted for inflammation using multipliers proposed by Thurnham et al. (2010)
\(^d\) Body iron calculation (mg/kg) = \(-[\log_{10}(sTfR \times 1000/ferritin) - 2.8229]/0.1207\) from Cogswell et al. (2009)
\(^e\) Odds ratio of Control relative to BLISS
\(^f\) Defined as plasma ferritin \(\geq 15\) \(\mu g/L\), in the absence of early functional iron deficiency, iron deficiency anaemia, or iron depletion
\(^g\) Defined as plasma ferritin <15 \(\mu g/L\), in the absence of early functional iron deficiency and iron deficiency anaemia
\(^h\) Defined as body iron <0 mg/kg and haemoglobin \(\geq 110\) g/L
\(^i\) Defined as body iron <0 mg/kg and haemoglobin <110 g/L
4.4 Discussion

There were no significant differences observed in the iron intake or biochemical iron status of infants following this baby-led approach (BLISS) to complementary feeding compared to those of infants following traditional spoon-feeding. However, our approach had been specifically modified to address concerns regarding the risk of iron deficiency. Furthermore, iron intakes were low in both groups at 7 months of age, with 74% of infants at risk of having inadequate intakes, and 17% had suboptimal iron status at 12 months. These findings illustrate that iron remains a nutrient of concern, regardless of the method of complementary feeding.

Although many parents are choosing to follow BLW with their infants (Cameron et al., 2013; Brown et al., 2011a; D’Andrea et al., 2016), we know almost nothing about what these infants are eating, and how this might impact their health. Only one small observational study has evaluated dietary intake in infants following unmodified BLW compared with age- and sex-matched infants following traditional spoon-feeding (Morison et al., 2016). In that study, despite similar energy intakes, BLW infants had significantly lower intakes of iron than traditionally spoon-fed infants (1.6 mg/day vs 3.6 mg/day, \( p<0.001 \)). By contrast, we found no difference in iron intake in our study between BLISS infants and Control infants. In fact, our BLISS infants were consuming a median of 3.0 mg of iron per day, suggesting that encouraging the intake of ‘high-iron’ foods as part of a baby-led approach to complementary foods was effective in improving iron intakes.

Our BLISS intervention recommended that ‘high-iron’ foods, particularly red meat and iron-fortified infant cereal, should be offered at every meal, from the start of the complementary feeding period. Red meat is high in bioavailable haem iron (Lombardi-Boccia et al., 2002) and a higher intake has been associated with higher serum ferritin (Szymlek-Gay et al., 2009), and haemoglobin (Olaya et al., 2013) concentrations in very young children. Similarly, iron-fortified infant cereal is high in iron and consumption in infants
has been shown to prevent iron deficiency anaemia (Walter et al., 1993). Significantly more BLISS infants were consuming red meat at 7 months than Control infants (76% vs 55%). This differs from the previous observational study which indicated that 6-8 month old infants following unmodified BLW and those following traditional spoon-feeding were similarly likely to consume red meat (58% of TSF vs 39% of BLW, \( p=0.225 \)) (Morison et al., 2016). However, it is important to note that only small amounts of red meat were being consumed by either group in the current study at 7 months. It is difficult to compare the exact amount of red meat consumed (in grams) in this study with other studies due to different ways of presenting data. For example, we presented the intakes of red meat as a total gram amount consumed per day, whereas other studies from high-income countries presented their data as an average portion size per eating occasion (Fox et al., 2006a; Mauch et al., 2015), or as a percentage of total iron intakes (Soh et al., 2001; Fox et al., 2006b; Wall et al., 2008). In the current study, we also reported the percentage of iron intakes from red meat, however, we split red meat from other meat (due to differences in iron content). Intake of red meat alone has only been reported in one other study of this age group. Those authors found that red meat intake contributed to 0% of iron intakes at 6-11 months and 2.4% at 12-24 months (Fox et al., 2006b). The latter finding reflects our Control participants at 12 months whose red meat intake contributed 2.5% of iron intakes. However, the BLISS group at 12 months, and both groups at 7 months, in the current study had a much higher contribution of red meat to total iron intakes than was reported in the Feeding Infants and Toddlers study (Fox et al., 2006b).

Iron-fortified infant cereal was introduced later in the BLISS group than in the Control group, but the difference was considerably smaller (BLISS 1.7 weeks later than Controls) than that observed in infants following unmodified BLW compared to age- and sex-matched spoon-fed Controls (5.1 weeks later (Morison et al., 2016)). At least part of the delay in introducing iron-fortified infant cereals seen in these two baby-led approaches (BLISS and unmodified BLW) is likely to be a reflection of later introduction to complementary foods overall, and possibly differences in the types of foods offered to these infants. As
was observed for red meat, the amount of iron-fortified infant cereal consumed was small in both groups. Wall et al. (2008) also found low intakes of fortified infant foods, which contributed to only 1% of iron intakes in infants aged 6-11 months and 0% of iron intakes between 12-24 months of age, although it was not specified what foods were considered ‘fortified infant foods’ in that study. In contrast, toddlers in the Feeding Infants and Toddlers study were consuming higher amounts of ‘infant cereal’ as their intakes contributed 7.5% of total iron intakes at 12-24 months (Fox et al., 2006b). Further research is required to determine whether a more intensive intervention could increase the amount of these important iron sources consumed, irrespective of whether the infant is following spoon-feeding or BLISS.

It is important to note that there has been some concern regarding dietary exposure to inorganic arsenic through infant rice cereals, and the potential health risks associated with high intakes in very young children (EFSA Panel on Contaminants in the Food Chain, 2009). There are currently no available data on arsenic concentrations in infant rice cereals which are available for purchase in New Zealand, or on the iron-fortified infant cereal which was provided as part of the intervention in the current study. We did not collect blood samples at 7 months of age, the age when consumption of iron-fortified infant cereal was highest. Very few participants in the current study were still consuming iron-fortified infant cereal ($n=11$), and only in very small quantities (consumers were consuming on average less than 10 g per day), at 12 months of age. However, even at 7 months of age, consumers were having on average less than 5 g per day of iron-fortified infant rice cereal. We did not analyse arsenic concentrations in the blood samples collected at 12 months of age, as arsenic is cleared quickly from the blood and therefore only reflects recent exposure (Klaassen et al., 1996). It appears unlikely that inorganic arsenic intakes would have caused any risk to the health of infants in the current study considering their low consumption (median intake <10 g per day). The World Health Organization (WHO) set a benchmark dose limit for a 0.5% increased incidence of lung cancer of 3.0 μg/kg body weight per day (World Health Organization, 2010). The European Food Safety Authority (EFSA)
calculated exposure to inorganic arsenic based on consumption of rice-based infant foods and reported that for every 90 g per day intake of rice based cereal for a 6 month old infant, exposure to inorganic arsenic was 1.63 μg/kg body weight per day (EFSA Panel on Contaminants in the Food Chain, 2009). The maximum average intake in the current study was 7.2 g per day. Based on the same estimates reported by EFSA, this would contribute only 0.13 μg/kg body weight per day, well below the benchmark dose limit of 3.0 μg/kg body weight per day (World Health Organization, 2010).

The only significant difference observed in the intake of an iron absorption modifier was the lower intake of Vitamin C (enhancer of iron absorption) at 7 months of age in BLISS compared with Control infants. This is consistent with the tendency of BLISS infants to consume a smaller amount of vegetables at that age (p=0.06), and to be less likely to consume commercial infant foods (Williams-Erickson, 2015). Many commercial infant foods have ascorbic acid added to them as an antioxidant (Thomson et al., 2007), and to improve iron absorption (Fairweather-Tait, 1989; Davidsson et al., 2000). The current intakes are lower (7 months: 49-59 mg/day, 12 months: 48-50 mg/day) than have been previously reported in New Zealand infants (62-90 mg/day) and toddlers (58-67 mg/day) (Soh et al., 2001; Wall et al., 2008), although in both these studies a wider age range of infants (6-11 months) and toddlers (12-24 months) were included. Although Vitamin C is an important enhancer of non-haem iron absorption (Institute of Medicine, 2002), a difference of approximately 10 mg per day is unlikely to be clinically important. It has been estimated that a ratio by weight of ascorbic acid (Vitamin C) to iron of 6:1 contributes a useful increase in non-haem iron absorption (Hurrell, 2002). The ratio of ascorbic acid to iron in the BLISS group at 7 months of age was 16:1 (49.2 mg Vitamin C/3.0 mg Iron, Table 4.4), and increasing the ascorbic acid intake by 10 mg per day would only increase this ratio to 19:1 ((49.2 mg + 9.7 mg Vitamin C)/3.0 mg iron, Table 4.4), an improvement of just 3:1. We did not measure iron status at 7 months, and therefore could not use this to determine the potential negative impact of the lower Vitamin C intake reported. However, given neither Vitamin C intake nor iron status were significantly different at 12
months of age, the difference we observed at 7 months seems unlikely to be important.

Several other food components in the diet can affect the absorption of iron, including enhancers (‘meat, fish, poultry’) and inhibitors (phytate, tannins). Although we were not able to assess whether there was any impact of these enhancers or inhibitors on the infants’ iron status, there was no difference in intakes of phytate or ‘meat, fish, poultry’ between groups. Few (n=2) participants in the current study drank tea, the highest source of tannins likely to be consumed in this population. This is somewhat lower than the number of infants and toddlers consuming tea in previous New Zealand studies. A study by Wall et al. (2008) reported that nine (3%) of their infant participants consumed tea, but was also not able to comment on whether this tea consumption impacted on their iron status. Grant et al. (2003), however, reported no increased risk of iron deficiency in a larger number of infants and toddlers (n=16, 5%) consuming tea (RR: 2.00; 95% CI: 0.50, 4.89) (Grant et al., 2003).

Only one participant in the current study consumed cow’s milk as a drink at 7 months of age. While they consumed more than the recommended amount for this age group (0 mL per day, for 0-12 months of age), they consumed less than the recommended amount of cow’s milk for infants 12 months of age (<500 mL per day) (Ministry of Health, 2008). High consumption of cow’s milk (≥500 mL per day) has been shown to be associated with lower ferritin concentrations in very young children (Daly et al., 1996; Soh et al., 2004; Gunnarsson et al., 2004; Uijterschout et al., 2014; Domellöf et al., 2014; Bramhagen, 1999). This association is largely because cow’s milk is low in iron and high intakes may displace other solid foods in the diet, reducing the intake of key nutrients, including iron (Ministry of Health, 2008) and high cow’s milk intake is associated with increased gastrointestinal blood loss in infants (Wilson et al., 1974; Ziegler, 1990). Although there was no difference between groups in the number consuming cow’s milk as a drink at 12 months of age, a relatively high proportion of participants consumed ≥500 mL per day (n=11, 8%).

The high proportion (74% of both groups) of infants at risk of inadequate iron intakes at 7 months of age compared to much lower proportions at 12
months of age (23% of Controls, 26% of BLISS) is in contrast with previous New Zealand data, which found 15% of infants (aged 6-11.9 months) and 66% of toddlers (aged 12-24 months) were at risk of inadequate iron intakes (Soh et al., 2001). However, that study used the EAR cut-point method (using the United Kingdom EAR values) rather than the full probability approach that was used in the current study, making it difficult to directly compare these results. In Europe, the risk of inadequate iron intakes also appears high (~50% of infants aged 6-12 months and ~60% of children aged 12-36 months) (Eussen et al., 2015) and comparable to our findings, although the EAR cut-point method was used to determine those prevalences. Unfortunately, we do not have a measure of iron status at 7 months to determine the impact of having such a high prevalence of inadequate intakes. However, at 12 months of age the risk of inadequate intakes was still relatively high, whereas our biochemical data show that 17% of infants in both groups had poor iron status, making it unlikely that our intakes are of major concern at 7 months. Alternatively, it may be that the cut offs for determining the risk of inadequate iron intakes needs reassessing. This is particularly so for younger infants, as there is no specific cut off for infants less than 8 months of age using the Institute of Medicine probabilities of inadequacy (Institute of Medicine, 2002).

Interestingly, our median plasma ferritin concentrations were higher in both groups (BLISS: 27.0 μg/L, Control: 28.9 μg/L) than in previous studies in very young children (18-20 μg/L) (Soh et al., 2004; Grant et al., 2007). As we had few participants with raised inflammatory markers, and adjusted our plasma ferritin concentrations for inflammation, the higher values seen in our study are not likely to be due to inflammation. These differences may reflect the higher numbers of infants in the current study consuming iron-fortified infant milks (33% of infants fed formula rather than breast milk at 12 months) than has been reported in the past (17% only formula fed) (Soh et al., 2001).

We did not reach our planned sample size of 168 blood samples, which would have allowed us to detect a difference in plasma ferritin concentrations between groups of 5.0 μg/L with 80% power at \( p < 0.05 \) (Daniels et al., 2015). Although 206 participants were recruited for the full study, only 119 (58%)
blood samples were collected. This was because, 22 participants withdrew from the study before 12 months, 13 participants were unable to be contacted or were living out of town, 26 blood draws were unsuccessful, and 26 participants refused the blood test, leaving the final sample of 119. Although this meant that the study sample was not powered to accept the null hypothesis, the confidence interval can tell us the range of plausible values for the difference between the two groups in the population. The most extreme difference consistent with the data was -10.9 μg/L (i.e. the lower confidence limit for the difference). This suggests that, in response to a BLISS intervention, the Control group’s median plasma ferritin concentration (28.9 μg/L) might, at most, fall to 18.0 μg/L – a value above the cut offs usually associated with deficiency (i.e. 12 or 15 μg/L). The data are also consistent with plasma ferritin rising to 34.7 μg/L (applying the upper confidence limit of 5.8 μg/L).

A higher cut off (<15 μg/L) was used to define depleted iron stores in the current study than is recommended by the WHO (<12 μg/L) (World Health Organization, 2001). This cut off was used because it is the cut off that was used clinically by the IANZ (International Accreditation New Zealand) accredited SCL that analysed the samples, and because no participant was classified as having depleted iron stores using the recommended cut off of <12 μg/L. This was surprising because studies have consistently demonstrated a pyramid type distribution of iron status with iron depletion more common than early functional iron deficiency which in turn is more common than iron deficiency anaemia (Looker et al., 1997; Karr et al., 1996; Soh et al., 2004). It appears that the use of body iron and haemoglobin to classify toddlers as early functional iron deficient or iron deficiency anaemic meant that those who would usually have been defined as iron depleted using a ferritin cut off of <12 μg/L, were instead classified to one of those stages of deficiency. The cut off of <15 μg/L used to define iron depletion in the current study gave a proportion of infants with depleted iron stores (5% of Control infants) that was similar to that in an observational study of Italian infants (6.2%) where the same cut off was used (Capozzi et al., 2010). While a study using NHANES survey 2003-2006 data also
defined iron deficiency using body iron, they did not report on the prevalence of depleted iron stores in this age group (Cogswell et al., 2009).

Only one other study has reported body iron concentration in New Zealand toddlers, reporting a mean concentration of 2.8 mg/kg (Szymlek-Gay et al., 2009), which is similar to our mean of 3.3 mg/kg. The number of infants with body iron less than 0 mg/kg (12% of Control and 14% of BLISS participants) was the same as has been reported for 1-2 year old children in the NHANES survey 2003-2006 (14%) (Cogswell et al., 2009), which was the first known study to describe iron deficiency using body iron in this age group. A similar percentage of infants in the Control and BLISS groups of the current study were classified as iron deficient anaemic (7% BLISS, 5% Control) to previous studies in New Zealand infants aged between 6 and 24 months, where prevalences of 4% (Soh et al., 2004) and 6% (Grant et al., 2007) have been reported.

A relatively high proportion of infants in both groups (13% BLISS, 10% Control) had a type of anaemia other than iron deficiency anaemia. Other types of anaemia may be caused by infection (malaria, HIV, and hookworm), folate or Vitamin B12 deficiency (macrocytic anaemia), or genetic disorders such as thalassemia and sickle cell anaemia (World Health Organization, 2001). The most likely cause in this population group would be folate or Vitamin B12 deficiency, as the other causes are usually found in low income countries (malaria) or in those of non-New Zealand European ethnicities such as African or Asian (thalassemia and sickle cell anaemia), which were not well represented in this study sample. However, no participant in the current study had a mean cell volume of greater than the upper level of the reference range of 86 fl (Southern Community Laboratories Ltd, 2014), that would be indicative of megaloblastic anaemia. This suggests that the cause was not likely due to another nutrient deficiency of folate or Vitamin B12. An alternative explanation for the high proportion of other anaemia could be the cut off used for defining anaemia. We used a haemoglobin concentration of <110 g/L to define anaemia, as this cut off is widely recommended in research and clinically (World Health Organization, 2001; Southern Community Laboratories Ltd, 2014). This value
was however extrapolated from older age groups (Gibson, 2005c), and there has been some discussion of whether a lower cut off may be more appropriate in this age group (Domellöf et al., 2002).

The current study suggests that when parents are given advice to offer infants ‘high-iron’ foods with every meal, total iron intake is similar to that of Control infants, and there is no difference in their iron status. These findings are important given concerns from health professionals that baby-led approaches to complementary feeding may increase the risk of iron deficiency (Cameron et al., 2012b; D'Andrea et al., 2016), and the observation that infants following unmodified BLW have significantly lower iron intakes (Morison et al., 2016).

Our study has a number of strengths including being the first randomised controlled trial to investigate the impact of a baby-led approach to complementary feeding on iron intake and biochemical iron status. We collected robust dietary intake data using three non-consecutive days of WDRs. As infants often do not eat all of the food offered to them we asked parents to weigh the food before and after eating (including food that was no longer on the surface on which it was originally offered) to ensure we had an accurate representation of actual consumption. Values for the haem iron and phytate content of foods were calculated for all raw and cooked foods, and recipes were disaggregated so that ingredients in recipes could be assigned to individual food groups rather than being reported as ‘mixed dishes’. This is beneficial for food group analyses as it prevented over- or under-estimation of foods consumed by infants within recipes, or mixed dishes. This was particularly important for the BLISS group who were specifically guided to make recipes, which included ‘high-iron’ foods such as iron-fortified infant cereal and red meat. We were also able to minimise any effect of inflammation or infection on plasma ferritin concentrations by waiting until the child was well before collecting the blood sample.

Limitations include the use of estimated breast milk volumes. This approach is commonly used when other methods are not feasible (Skinner et al., 1997; Devaney et al., 2004; Ponza et al., 2004; Wall et al., 2008; Briefel et al., 2010; Sharma et al., 2013) but does mean that we do not have specific intake values for individuals. It must be noted that although we used WDRs
(considered to provide the best estimate of dietary intakes in very young children) to collect dietary intake data, there is a possibility this has led to some inaccuracy, particularly because of participant burden (participants were asked to complete 3-day WDRs at both 7 and 12 months). Participants may have changed the types of foods offered to their child on recording days due to ease of recording, and beliefs as to what is considered healthy or unhealthy. Although our study was designed to detect differences of 5.0 μg/L in geometric mean plasma ferritin concentrations with 168 participants, blood samples were only obtained from 119 participants, which influenced our power to detect statistically significant differences. However, we have reported confidence intervals so the reader can see the range of plausible differences. Lastly, we used body iron for determining iron status categories which has been validated for use in adults (Cook et al., 2003). Whether the body iron calculation is an acceptable measure for use in infants and children is not known, although it has been used in this age group in other studies, including the analysis of NHANES data (Cogswell et al., 2009).

In summary, there was no evidence of a difference in iron intakes and iron status between infants fed using traditional spoon-feeding and infants following this modified version of BLW in which parents were given advice to offer ‘high-iron’ foods with each meal, suggesting that a baby-led approach can be used without impacting negatively on iron status. However, it is important to note that this study assessed a modified version of BLW so no conclusions can be made about the risk of iron deficiency in infants following traditional BLW. In addition, iron intakes were low for most infants at 7 months, and iron status was low for one in six infants at 12 months. These findings highlight that work is still needed to improve the iron intake and iron status of infants whether they are following BLISS or traditional spoon-feeding.
An abstract based on aspects of this chapter has been published and was presented at the European Academy of Paediatric Societies 2016 conference

5 Impact of a baby-led approach to complementary feeding on zinc intake and status

6-month-old Quinn tries some red meat (a good source of bioavailable zinc)
5.1 Introduction

A baby-led approach to complementary feeding may affect the dietary zinc intakes of infants, as there is often an emphasis on fruit and vegetables (Brown et al., 2011a), which are easy foods for infants to hold and feed themselves but are low in bioavailable zinc (Brown et al., 2004). Zinc intakes may also be lower if other whole foods, such as meat, that are high in bioavailable zinc (Brown et al., 2004) are not offered because parents are concerned about choking (Cameron et al., 2012b; D’Andrea et al., 2016). This would be a concern because complementary foods are a critical source of zinc from 6 months of age, by which time breast milk no longer provides sufficient zinc (World Health Organization, 2004b). Inadequate zinc status in very early childhood has been associated with an increased risk of infection (Shankar et al., 1998; Fraker et al., 2000), and impaired growth (Nissensohn et al., 2016).

Surprisingly, given the interest in Baby-Led Weaning (BLW) amongst parents, to date, only one study has compared the dietary intakes of infants following BLW with infants following a more traditional spoon-feeding approach to complementary feeding (Morison et al., 2016). In this small study, 6-8 month old infants who were following a BLW style of complementary feeding had significantly (by 21%) lower zinc intakes than their age- and sex-matched counterparts who were following traditional spoon-feeding (Morison et al., 2016). As infants and toddlers from high-income countries may already be at risk of mild zinc deficiency (Morgan et al., 2010; Krebs et al., 2006), it is important to understand what the key food sources of zinc are during the complementary feeding period, and how adequate zinc intakes and biochemical status are, both for infants following traditional spoon-feeding and for those following baby-led approaches to complementary feeding.

The objective addressed in this chapter was to determine the zinc intake (at 7 and 12 months of age) and zinc status (at 12 months of age) of infants following BLISS compared with those of infants following traditional spoon-feeding.
5.2 Methods

5.2.1 Data collection
Refer to Chapter 3 for a full description of the methods used to collect data for the BLISS study. Briefly, 206 participants were randomised to Control or BLISS groups. Both groups received standard midwifery and Well Child care from before birth. The BLISS group received eight additional visits (from before birth to 9 months of age) providing education and support on following the BLISS approach to complementary feeding (i.e. Baby-Led Weaning modified to address concerns about iron, growth, and choking). Weighed three-day diet records (WDRs) were used to assess the intake of zinc and phytate at 7 and 12 months of age (Chapter 3, Section 3.8), and a blood sample was collected to determine plasma zinc concentrations at 12 months of age (Chapter 3, Section 3.10).

5.2.2 Statistical analysis
The zinc intake and status analyses were secondary outcomes of the BLISS study. The data were analysed according to modified intention-to-treat and all analyses were conducted using Stata, version 14.2 (StataCorp LP, Texas, US). Note that some analyses were very similar to analyses presented in the iron chapter (Chapter 4).

The characteristics of mothers and infants who provided intake data at 7 or 12 months of age, or blood test data at 12 months of age were compared to those of participants who did not provide data using two-sample t-tests (continuous variables) or chi-squared tests (categorical variables). The proportions of infants at 7 and 12 months of age fed breast milk, infant formula, or breast milk and infant formula, as their infant milk, as well as those consuming cow’s milk as a drink, were determined using Chi-squared tests.

All nutrient and food group data were presented as daily averages over the three days of WDR collection. As the median intakes of some food groups were very small (e.g., median intake of ‘legumes’ = 0.0 g), these food groups
were collapsed with other small food groups of similar composition as follows: 1) ‘legumes’, and ‘nuts and seeds’ were combined; 2) ‘cow’s milk’ and ‘dairy’ were combined; 3) ‘beverages’ and ‘miscellaneous’ were combined; and 4) ‘fish and poultry’ and ‘other meat’ were combined. Combinations 1 and 2 were made because the food groups were considered to be similar and to be good sources of protein and relatively good sources of zinc when compared with ‘fruit and fruit juice’ and ‘vegetables’. Combination 4 was made because these food groups were considered to be higher sources of zinc than food groups in combinations 1 and 2, but lower than ‘red meat’.

Medians and lower and upper quartiles (25th and 75th) were reported because the data were skewed. Quantile regression was used to estimate group differences in the intake of energy, nutrients, and food components (e.g., zinc, phytate), adjusted for infant age and sex, maternal education (non-tertiary vs tertiary), and maternal parity (1 child vs >1 child, including the current pregnancy). The contribution of zinc (mg/food group/day) and percentage of zinc from each of the food groups were also determined using regression analysis with adjustments as above.

‘Usual’ zinc intake was determined, as three days of dietary data do not provide a good estimate of ‘usual’ dietary intakes (Harttig et al., 2011). As described in Chapter 4, data from each participant’s WDR was imported into the Multiple Source Method web-based programme (Harttig et al., 2011). The programme calculated the ‘usual’ intake of zinc for each participant by accounting for intra-individual variation (Harttig et al., 2011). Using the ‘usual’ intake data the prevalence and odds ratios for inadequate intakes were calculated based on the number of participants at 7 and 12 months with an intake below the Estimated Average Requirement (EAR) of 2.5 mg/day (Department of Health and Ageing Australia et al., 2006; Institute of Medicine, 2002), as well as those above the Upper Intake Level (UL) of 5 mg/day at 7 months of age and 7 mg/day at 12 months of age (Department of Health and Ageing Australia et al., 2006; Institute of Medicine, 2002).

Mean plasma zinc concentrations were adjusted for infant age and sex, and maternal education and parity (as above). These adjustment factors were
decided *a priori*. Presence of inflammation (based on C-reactive protein (CRP) >5 mg/L and α1-acid glycoprotein (AGP) >1 g/L), time of sample collection, and time since last meal were also controlled for as these factors were considered likely to affect plasma zinc concentrations (IZiNCG, 2007).
5.3 Results

5.3.1 Participant characteristics

The baseline data presented here are the same as the baseline data presented in the iron chapter (Chapter 4). They have been included in this chapter for completeness. A total of 206 infants participated in the BLISS study (Chapter 3, Figure 3.1). Baseline demographic data are shown in Table 5.1 for participants who provided either a WDR (intake data) or biochemical sample (status data) (n=169, 82%). Participants who provided data were similar to those who did not for all variables shown, with the exception of maternal age (which was higher for those who provided data; Table 5.2). The mean age of parent participants was 32 years when their infant was born, 41% were primiparous, 83% were New Zealand European, and 51% had a university qualification. The level of household deprivation was high for 22% of participants (compared to 30% for the New Zealand population (Atkinson et al., 2014)).
Table 5.1 Characteristics of participants who provided intake data at 7 ($n=162$) and/or 12 ($n=143$) months of age or biochemical data at 12 ($n=119$) months of age$^a$

<table>
<thead>
<tr>
<th>Maternal and household variables</th>
<th>Control ($n=81$)</th>
<th>BLISS ($n=88$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at birth, years [mean (SD)]</td>
<td>32.2 (5.8)</td>
<td>31.7 (4.8)</td>
</tr>
<tr>
<td>Maternal parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First child</td>
<td>32 (40)</td>
<td>37 (42)</td>
</tr>
<tr>
<td>Two children</td>
<td>27 (33)</td>
<td>37 (42)</td>
</tr>
<tr>
<td>Three or more children</td>
<td>22 (27)</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Maternal ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>70 (87)</td>
<td>71 (80)</td>
</tr>
<tr>
<td>Māori</td>
<td>6 (7)</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (6)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>School only</td>
<td>23 (28)</td>
<td>26 (30)</td>
</tr>
<tr>
<td>Post-secondary</td>
<td>13 (16)</td>
<td>20 (22)</td>
</tr>
<tr>
<td>University</td>
<td>45 (56)</td>
<td>42 (48)</td>
</tr>
<tr>
<td>Household deprivation$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 (Low)</td>
<td>24 (30)</td>
<td>25 (28)</td>
</tr>
<tr>
<td>4-6</td>
<td>37 (45)</td>
<td>46 (53)</td>
</tr>
<tr>
<td>7-10 (High)</td>
<td>20 (25)</td>
<td>17 (19)</td>
</tr>
<tr>
<td>Infant variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>37 (46)</td>
<td>50 (57)</td>
</tr>
<tr>
<td>Male</td>
<td>44 (54)</td>
<td>38 (43)</td>
</tr>
<tr>
<td>Infant birth weight, g [mean (SD)]</td>
<td>3510 (453)</td>
<td>3496 (448)</td>
</tr>
<tr>
<td>Infant gestational age at birth, weeks [mean (SD)]</td>
<td>39.5 (1.2)</td>
<td>39.7 (1.0)</td>
</tr>
</tbody>
</table>

Abbreviations: NZ European, New Zealand European

$^a$Data presented as $n$ (%), unless otherwise stated

$^b$Household deprivation categorised using the NZDep2013 scale in which decile 1 indicates the lowest level of deprivation and 10 indicates the highest (Atkinson et al., 2014)
Table 5.2 Comparisons of those included (provided either intake or status data) and not included (provided neither intake nor status data) in this data set

<table>
<thead>
<tr>
<th>Maternal and household variables</th>
<th>Included (n=169)</th>
<th>Not included (n=37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at birth, years [mean (SD)]</td>
<td>31.9 (5.3)</td>
<td>28.3 (5.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal parity</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First child</td>
<td>69 (41)</td>
<td>16 (43)</td>
<td></td>
</tr>
<tr>
<td>Two children</td>
<td>64 (38)</td>
<td>11 (30)</td>
<td></td>
</tr>
<tr>
<td>3 or more children</td>
<td>36 (21)</td>
<td>10 (27)</td>
<td></td>
</tr>
<tr>
<td>Maternal ethnicity</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>141 (83)</td>
<td>27 (73)</td>
<td></td>
</tr>
<tr>
<td>Māori</td>
<td>14 (8.5)</td>
<td>6 (16)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>14 (8.5)</td>
<td>4 (11)</td>
<td></td>
</tr>
<tr>
<td>Maternal education</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>School only</td>
<td>49 (29)</td>
<td>14 (38)</td>
<td></td>
</tr>
<tr>
<td>Post-secondary</td>
<td>33 (20)</td>
<td>10 (27)</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>87 (51)</td>
<td>13 (35)</td>
<td></td>
</tr>
<tr>
<td>Household deprivation</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 (Low)</td>
<td>49 (29)</td>
<td>11 (30)</td>
<td></td>
</tr>
<tr>
<td>4-6</td>
<td>83 (49)</td>
<td>19 (51)</td>
<td></td>
</tr>
<tr>
<td>7-10 (High)</td>
<td>37 (22)</td>
<td>7 (19)</td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2 (continued) Comparisons of those included (provided either intake or status data) and not included (provided neither intake nor status data) in this data set

<table>
<thead>
<tr>
<th>Infant variables</th>
<th>Included (n=169)</th>
<th>Not included (n=37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>87 (51)</td>
<td>22 (61)</td>
<td>0.29</td>
</tr>
<tr>
<td>Male</td>
<td>82 (49)</td>
<td>14 (39)</td>
<td></td>
</tr>
<tr>
<td>Infant birth weight, g [mean (SD)]</td>
<td>3503 (449)</td>
<td>3619 (545)</td>
<td>0.18</td>
</tr>
<tr>
<td>Infant gestational age at birth, weeks [mean (SD)]</td>
<td>39.6 (1.1)</td>
<td>39.6 (1.0)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Bold indicates a statistically significant difference at p<0.05

Abbreviations: NZ European, New Zealand European

Data presented as n (%), unless otherwise stated

Household deprivation categorised using the NZDep2013 scale in which decile 1 indicates the lowest level of deprivation and 10 indicates the highest (Atkinson et al., 2014)
5.3.2 Adherence to a baby-led approach and to delaying the introduction of complementary foods

Infants in the BLISS group were more adherent to a baby-led approach to complementary feeding than Controls, with 74% feeding themselves most or all of their food in the past week compared to 19% of Controls ($p<0.001$) at 7 months, and 77% compared to 48% ($p<0.001$) at 12 months. More BLISS infants than Control infants reached 6 months of age before complementary foods were introduced (66% vs 18%; $p<0.001$); BLISS infants were 24.6 weeks of age on average compared to 22.6 weeks for Control infants when complementary foods were introduced ($p<0.001$).

5.3.3 Infant milk intakes

There was no difference between groups in the type of infant milk consumed during the three day WDR at either 7 or 12 months of age ($p=0.95$ and $p=0.94$, respectively). At 7 months, 51% of all infants were fed breast milk, 24% were fed infant formula (fortified with zinc (between 3-6 mg per 100 g powder) and iron (between 4-10 mg per 100 g powder)), and 25% were fed both infant formula and breast milk. At 12 months, 43% of all infants were fed breast milk, 33% were fed infant formula, 11% were fed both infant formula and breast milk, and 13% were not fed either infant milk. The estimated median amount of breast milk and measured amount of infant formula consumed was also similar between groups at 7 (estimated breast milk: 750 g Control, 750 g BLISS, $p=1.00$; measured infant formula: 309 g Control, 525 g BLISS, $p=0.17$) and 12 (estimated breast milk: 448 g Control, 448 g BLISS, $p=0.94$; measured infant formula: 414 g Control, 329 g BLISS, $p=0.38$) months.
5.3.4 Zinc and phytate intakes

Zinc and phytate intakes were similar for the Control and BLISS groups at both 7 and 12 months (Tables 5.3 and 5.4), although there was a tendency for a higher phytate-to-zinc molar ratio in BLISS infants compared with Control infants at 7 months ($p=0.06$). The majority of zinc intakes were still provided by breast milk or infant formula at 7 months, with complementary foods only providing, on average, 24% (0.59 mg/day) and 29% (0.72 mg/day) of the EAR for zinc (2.5 mg/day) for the Control and BLISS groups, respectively (Table 5.3). By 12 months of age, this had risen to 104% (2.6 mg/day) for the Control group and 116% (2.9 mg/day) for the BLISS group (Table 5.4).

The prevalence of inadequate zinc intakes was low at 7 months: 9% of Control and 5% of BLISS infants. At 12 months, even fewer (1% in each group) had inadequate zinc intakes. There was no evidence of a difference in the odds of inadequate zinc intakes between the groups at either 7 (OR: 0.5; 95% CI: 0.1, 1.8; $p=0.28$) or 12 (OR: 0.9; 95% CI: 0.1, 14.8; $p=0.94$) months. In contrast, the prevalence of zinc intakes above the UL was 23% at 7 months, and 4% at 12 months, although, there was no significant difference between groups at either 7 (OR: 1.0; 95% CI: 0.5, 2.1; $p=0.98$) or 12 (OR: 0.9; 95% CI: 0.2, 4.6; $p=0.90$) months. No participants were taking supplements containing zinc.
Table 5.3 Intake of zinc and phytate at 7 months of age from complementary foods and infant milks\textsuperscript{a,b}

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BLISS</th>
<th>Difference (95% CI)\textsuperscript{c}</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 months of age</td>
<td>n=77</td>
<td>n=85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kJ/day [mean (SD)]</td>
<td>2862 (548)</td>
<td>2996 (613)</td>
<td>145 (-31.2, 321)</td>
<td>0.11</td>
</tr>
<tr>
<td>Energy from complementary foods only, kJ/day [mean (SD)]\textsuperscript{d}</td>
<td>672 (506)</td>
<td>799 (595)</td>
<td>144 (-26.2, 314)</td>
<td>0.10</td>
</tr>
<tr>
<td>Dietary zinc, mg/day</td>
<td>3.5 (2.7, 4.8)</td>
<td>3.5 (2.9, 4.7)</td>
<td>0.26 (-0.4, 0.9)</td>
<td>0.42</td>
</tr>
<tr>
<td>Dietary zinc from complementary foods only, mg/day</td>
<td>0.59 (0.29,1.3)</td>
<td>0.72 (0.47, 1.2)</td>
<td>0.14 (-0.1, 0.4)</td>
<td>0.24</td>
</tr>
<tr>
<td>Phytate, mg/day</td>
<td>35.6 (16.3, 75.2)</td>
<td>45.5 (23.0, 77.6)</td>
<td>4.2 (-15.0, 23.4)</td>
<td>0.67</td>
</tr>
<tr>
<td>Phytate:zinc molar ratio\textsuperscript{e}</td>
<td>0.92 (0.46, 2.1)</td>
<td>1.4 (0.58, 2.0)</td>
<td>0.43 (-0.0, 0.9)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Median (25\textsuperscript{th}, 75\textsuperscript{th} percentile), unless otherwise stated  
\textsuperscript{b}Intake reported in the three day weighed diet records collected at 7 months of age  
\textsuperscript{c}Difference adjusted for infant age and sex, maternal education and parity  
\textsuperscript{d}Excludes intake from breast milk and infant formula  
\textsuperscript{e}Calculated as [phytate (mg) / 660] / [zinc (mg) / 65.4]
Table 5.4 Intake of zinc and phytate at 12 months of age from complementary foods and infant milks\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>12 months of age</th>
<th>Control</th>
<th>BLISS</th>
<th>Difference (95% CI)\textsuperscript{c}</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n=68 )</td>
<td>( n=75 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kJ/day [mean (SD)]</td>
<td>3573 (776)</td>
<td>3623 (1048)</td>
<td>109 (-191, 409)</td>
<td>0.48</td>
</tr>
<tr>
<td>Energy from complementary foods only, kJ/day</td>
<td>2400 (848)</td>
<td>2527 (1183)</td>
<td>195 (-142, 533)</td>
<td>0.25</td>
</tr>
<tr>
<td>Dietary zinc, mg/day</td>
<td>4.4 (3.6, 5.7)</td>
<td>4.4 (3.8, 5.3)</td>
<td>-0.05 (-0.6, 0.5)</td>
<td>0.86</td>
</tr>
<tr>
<td>Phytate, mg/day</td>
<td>2.6 (2.0, 3.8)</td>
<td>2.9 (2.2, 3.7)</td>
<td>0.27 (-0.2, 0.8)</td>
<td>0.29</td>
</tr>
<tr>
<td>Phytate:zinc molar ratio\textsuperscript{e}</td>
<td>187 (118, 310)</td>
<td>229 (152, 274)</td>
<td>37.2 (-20.4, 94.8)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Median (25\textsuperscript{th}, 75\textsuperscript{th} percentile), unless otherwise stated  
\textsuperscript{b}Intake reported in the three day weighed diet records collected at 12 months of age  
\textsuperscript{c}Difference adjusted for infant age and sex, maternal education and parity  
\textsuperscript{d}Excludes intake from breast milk and infant formula  
\textsuperscript{e}Calculated as [phytate (mg) / 660] / [zinc (mg) / 65.4]
5.3.5 Complementary food sources of zinc

While there were no group differences in the total amount of zinc consumed per day, several differences were observed in the food sources of zinc.

At 7 months, breast milk and infant formula were the predominant sources of zinc in both groups, but BLISS infants obtained significantly more zinc from ‘breads and cereals’, ‘dairy’, ‘red meat’, ‘miscellaneous’, ‘eggs’, and ‘legumes, nuts and seeds’ than Control infants, and significantly less zinc from ‘vegetables’ (Table 5.5). The food group contributing the most to zinc intakes at 7 months was ‘vegetables’ in the Control group (18% of zinc intake from complementary foods), and ‘breads and cereals’ in the BLISS group (20% of zinc intake from complementary foods) (Table 5.5).

By 12 months, complementary foods were the predominant sources of zinc intakes with around one third coming from infant milks in both groups. Most of the differences seen at 7 months were no longer visible at 12 months except that ‘vegetables’ continued to contribute significantly less to the zinc intakes of BLISS infants than Control infants, although the difference was small (median difference: 0.07 mg/day, \( p=0.041 \)) (Table 5.6). Both Control and BLISS infants consumed most of their zinc from ‘dairy’ (24% and 27% of zinc from complementary foods, respectively) (Table 5.6).
**Table 5.5** Contribution of food groups (other than infant milks) to zinc intake at 7 months of age\(^{a,b,c}\)

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Control mg/day</th>
<th>%d</th>
<th>BLISS mg/day</th>
<th>%d</th>
<th>Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7 months of age</strong></td>
<td>n=77</td>
<td></td>
<td>n=85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td>0.12 (0.0, 0.2)</td>
<td>18 (11, 32)</td>
<td>0.07 (0.0, 0.1)</td>
<td>9.8 (6, 16)</td>
<td>-0.05 (-0.1, -0.0)</td>
<td>0.025</td>
</tr>
<tr>
<td>Breads and cereals</td>
<td>0.07 (0.0, 0.2)</td>
<td>12 (4, 26)</td>
<td>0.18 (0.1, 0.3)</td>
<td>20 (15, 28)</td>
<td>0.10 (0.1, 0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>0.07 (0.0, 0.1)</td>
<td>9.4 (4, 18)</td>
<td>0.05 (0.0, 0.1)</td>
<td>5.4 (3, 11)</td>
<td>-0.02 (-0.1, 0.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Dairy</td>
<td>0.05 (0.0, 0.1)</td>
<td>9.0 (0, 17)</td>
<td>0.12 (0.0, 0.2)</td>
<td>19 (5, 29)</td>
<td>0.07 (0.0, 0.1)</td>
<td>0.012</td>
</tr>
<tr>
<td>Red meat(^f)</td>
<td>0.01 (0.0, 0.3)</td>
<td>8.5 (0, 26)</td>
<td>0.16 (0.0, 0.2)</td>
<td>18 (2, 31)</td>
<td>0.07 (0.0, 0.1)</td>
<td>0.039</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>0.0 (0.0, 0.1)</td>
<td>0.3 (0, 10)</td>
<td>0.0 (0.0, 0.01)</td>
<td>0.4 (0, 2)</td>
<td>0.00 (-0.0, 0.0)</td>
<td>0.61</td>
</tr>
<tr>
<td>Miscellaneous(^g)</td>
<td>0.0 (0.0, 0.01)</td>
<td>0.3 (0, 3)</td>
<td>0.01 (0.0, 0.02)</td>
<td>1.0 (0, 4)</td>
<td>0.01 (-0.0, 0.0)</td>
<td>0.035</td>
</tr>
<tr>
<td>Other meat(^h)</td>
<td>0.0 (0.0, 0.1)</td>
<td>0.0 (0, 10)</td>
<td>0.01 (0.0, 0.1)</td>
<td>0.6 (0, 7)</td>
<td>0.01 (-0.0, 0.0)</td>
<td>0.49</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.0 (0.0, 0.0)</td>
<td>0.0 (0, 0)</td>
<td>0.01 (0.0, 0.03)</td>
<td>0.2 (0, 1)</td>
<td>0.01 (0.0, 0.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Legumes, nuts and seeds</td>
<td>0.0 (0.0, 0.0)</td>
<td>0.0 (0, 0)</td>
<td>0.01 (0.0, 0.03)</td>
<td>0.8 (0, 5)</td>
<td>0.01 (0.0, 0.01)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**Bold** indicates a statistically significant difference at p<0.05

\(^{a}\)Median (25\(^{th}\), 75\(^{th}\) percentile)

\(^{b}\)Intake reported in the weighed three-day diet records collected at 7 months of age

\(^{c}\)Ordered by the food group contributing the most to zinc intakes in the Control group

\(^{d}\)Data expressed as median percentage contribution to total zinc intake from complementary foods (note: mean percentages added to 100% of total zinc intakes from complementary foods but are not reported here as the data are skewed)

\(^{e}\)Difference in median zinc (mg/day) intake between groups: negative values represent lower values in BLISS than in Control, positive values represent higher values in BLISS than in Control, adjusted for infant age and sex, maternal education and parity

\(^{f}\)Red meat: beef, lamb, mutton, venison

\(^{g}\)Miscellaneous: fats, sugar, sweet foods, herbs and spices, sauces, spreads, beverages etc.

\(^{h}\)Other meat: fish, poultry, pork, processed meats
Table 5.6 Contribution of food groups (other than infant milks) to zinc intake at 12 months of age\textsuperscript{a,b,c}

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Control</th>
<th>BLISS</th>
<th>Difference (95% CI)\textsuperscript{e}</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/day</td>
<td>%\textsuperscript{d}</td>
<td>mg/day</td>
<td>%\textsuperscript{d}</td>
</tr>
<tr>
<td>12 months of age</td>
<td>n=68</td>
<td>n=75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>0.59 (0.3, 1.1)</td>
<td>24 (13, 38)</td>
<td>0.73 (0.4, 1.4)</td>
<td>27 (13, 42)</td>
</tr>
<tr>
<td>Breads and cereals</td>
<td>0.52 (0.3, 0.7)</td>
<td>18 (12, 25)</td>
<td>0.67 (0.4, 0.8)</td>
<td>23 (15, 30)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>0.26 (0.1, 0.4)</td>
<td>8.9 (5, 13)</td>
<td>0.19 (0.1, 0.3)</td>
<td>6.4 (4, 10)</td>
</tr>
<tr>
<td>Other meat\textsuperscript{f}</td>
<td>0.24 (0.1, 0.5)</td>
<td>8.4 (2, 17)</td>
<td>0.17 (0.1, 0.4)</td>
<td>5.6 (2, 13)</td>
</tr>
<tr>
<td>Red meat\textsuperscript{g}</td>
<td>0.17 (0.0, 0.5)</td>
<td>5.6 (0, 20)</td>
<td>0.22 (0.0, 0.6)</td>
<td>9.4 (0, 22)</td>
</tr>
<tr>
<td>Fruit and fruit juice</td>
<td>0.11 (0.1, 0.2)</td>
<td>4.4 (3, 7)</td>
<td>0.16 (0.1, 0.2)</td>
<td>5.8 (3, 8)</td>
</tr>
<tr>
<td>Miscellaneous\textsuperscript{h}</td>
<td>0.09 (0.05, 0.3)</td>
<td>3.1 (2, 9)</td>
<td>0.09 (0.0, 0.2)</td>
<td>3.6 (1, 7)</td>
</tr>
<tr>
<td>Legumes, nuts and seeds</td>
<td>0.02 (0.0, 0.1)</td>
<td>0.7 (0, 3)</td>
<td>0.03 (0.0, 0.1)</td>
<td>1.0 (0, 4)</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.01 (0.0, 0.09)</td>
<td>0.2 (0, 3)</td>
<td>0.03 (0.0, 0.1)</td>
<td>1.2 (0, 3)</td>
</tr>
<tr>
<td>Iron-fortified infant cereal</td>
<td>0.0 (0.0, 0.0)</td>
<td>0.0 (0, 0)</td>
<td>0.0 (0.0, 0.0)</td>
<td>0.0 (0, 0)</td>
</tr>
</tbody>
</table>

\textbf{Bold} indicates a statistically significant difference at p<0.05
\textsuperscript{a}Median (25\textsuperscript{th}, 75\textsuperscript{th} percentile)
\textsuperscript{b}Intake reported in the weighed three-day diet records collected at 12 months of age
\textsuperscript{c}Ordered by the food group contributing the most to zinc intakes in the Control group
\textsuperscript{d}Data expressed as median percentage contribution to total zinc intake from complementary foods (note: mean percentages added to 100% of total zinc intakes from complementary foods but are not reported here as the data are skewed)
\textsuperscript{e}Difference in median zinc (mg/day) intake between groups: negative values represent lower values in BLISS than in Control, positive values represent higher values in BLISS than in Control, adjusted for infant age and sex, maternal education and parity
\textsuperscript{f}Other meat: fish, poultry, pork, processed meats
\textsuperscript{g}Red meat: beef, lamb, mutton, venison
\textsuperscript{h}Miscellaneous: fats, sugar, sweet foods, herbs and spices, sauces, spreads, beverages etc.
5.3.6 Biochemical zinc status

At 12 months of age, adjusted mean (SD) plasma zinc concentrations were 9.6 (1.5) μmol/L for the Control group and 9.6 (1.6) μmol/L for the BLISS group, and were not statistically significantly different (-0.09 μmol/L; 95% CI: -0.67, 0.48; p=0.75) (Table 5.7). Unadjusted plasma zinc concentrations were also not different between groups (-0.10 μmol/L; -0.67, 0.47; p=0.72). A high percentage of infants had low plasma zinc concentrations (using the IZiNCG criteria of <9.9 μmol/L (IZiNCG, 2007)) in both groups (63% of Control, 57% of BLISS) and there was no evidence of a difference between the groups (p=0.49) (Table 5.7). Very few participants had signs of acute or chronic inflammation (n=0 with raised CRP alone; n=2 Control and n=0 BLISS with raised CRP and raised AGP; n=6 Control and n=11 BLISS with raised AGP alone).

Table 5.7 Plasma zinc concentrations at 12 months of age

<table>
<thead>
<tr>
<th>12 months of age</th>
<th>Control</th>
<th>BLISS</th>
<th>Difference (95% CI)*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma zinc, μmol/L [mean (SD)]</td>
<td>n=57</td>
<td>n=58</td>
<td>-0.09 (-0.67, 0.48)</td>
<td>0.75</td>
</tr>
<tr>
<td>Plasma zinc &lt;9.9 μmol/Lb, [n (%)]</td>
<td>36 (63)</td>
<td>33 (57)</td>
<td>0.49</td>
<td></td>
</tr>
</tbody>
</table>

*a Adjusted for infant age and sex, maternal education and parity, acute phase protein status, time of sample collection, and time since last meal
b Reference level for low plasma zinc, morning non-fasting <9.9 μmol/L (IZiNCG, 2007)
5.4 Discussion

Infants following this modified version of BLW (BLISS) had similar total zinc and phytate intakes to infants following traditional spoon-feeding, and had comparable biochemical zinc status. However, the food sources of zinc differed at 7 months of age, with the largest contributor to the BLISS group’s zinc intake from complementary foods being ‘breads and cereals’, in contrast to the Control group for whom ‘vegetables’ was the predominant source. By 12 months of age, few differences were apparent, with both groups obtaining most of their zinc from complementary foods from the ‘dairy’ food group. Although only a small percentage of infants had inadequate zinc intakes at 7 (9% Control, 5% BLISS) and 12 (1% both groups) months, there was a high prevalence of low biochemical zinc status in both groups (63% Control, 57% BLISS) at 12 months.

Health professionals have expressed concerns that baby-led approaches to complementary feeding may increase the risk of iron deficiency (Cameron et al., 2012b; D’Andrea et al., 2016), particularly if foods low in iron are predominantly offered because they are easy to pick up (e.g., vegetables and fruit), and may be perceived to be less likely to cause choking than foods such as meat (Cameron et al., 2012b; D’Andrea et al., 2016). Given that iron and zinc are found in similar foods, this raises the concern that zinc intakes may also be inadequate in infants following a baby-led approach. As previously shown (Chapter 4), recommendations to provide ‘high-iron’ foods to infants following a baby-led approach to complementary feeding can offset the lower iron intakes observed in those following unmodified BLW (Morison et al., 2016). The small observational study of infants by Morison et al. (2016) found that infants aged 6-8 months following unmodified BLW also had significantly lower zinc intakes than age- and sex-matched infants following traditional spoon-feeding (3.7 vs. 3.0 mg/day, p=0.001), despite the absence of differences in energy intake (Morison et al., 2016). Both the previous study and the current study demonstrate that few infants fail to meet the EAR for zinc of 2.5 mg/day (Department of Health and Ageing Australia et al., 2006; Institute of Medicine,
However, unlike the previous study, the current study reported no differences in zinc intakes between the groups at either 7 or 12 months of age. BLISS participants were strongly advised to include ‘high-iron’ foods with every meal from the start of complementary feeding, particularly red meat, a food source that is rich in highly bioavailable zinc (Jalla et al., 2002). The current results suggest that such recommendations enable infants following a baby-led approach to complementary feeding to match the zinc intakes of infants fed via traditional spoon-feeding methods (average intake for both groups: 3.5 mg/day at 7 months, and 4.4 mg/day at 12 months), although biochemical zinc status remained as inadequate as that of the Controls.

Considering the adequacy of zinc intakes seen in this study, and the relative proximity of the EAR (2.5 mg/day) and Recommended Dietary Intake (RDI) (3 mg/day) to the UL (5 and 7 mg/day) (Department of Health and Ageing Australia et al., 2006; Institute of Medicine, 2002), it was possible that some infants were consuming intakes above the UL. Previous studies have reported that the percentage of infants (7-12 months) and toddlers (1-3 years) in high-income countries exceeding the UL for zinc intake is between 25 and 86% (Arsenault et al., 2003; Devaney et al., 2004; Hennessy-Priest et al., 2008; Butte et al., 2010; Rangan et al., 2015). In these studies, infants and toddlers were consuming zinc-fortified foods and supplements, as well as unfortified food sources of zinc. In contrast, in the current study, only 23% of infants at 7 months and 4% of infants at 12 months were above the UL of 5 mg/day for 7-12 months and 7 mg/day for 1-3 years. This is surprising because there was a very low prevalence of inadequate zinc intakes (7 months: 5-9%; 12 months: 1%), but may be explained by the finding that just 24% (7 months) and 33% (12 months) were consuming infant formula (fortified with between 2.9 mg and 5.8 mg zinc per 100 g) as their only infant milk, and no infants in either group were consuming zinc supplements.

There has been disagreement regarding an appropriate value for the UL across expert groups, and it has been suggested that the UL for children may be too low (Brown et al., 2004; Bertinato et al., 2013). This may certainly be the case for infants and toddlers as well as children, since it is not clear where their
UL should lie between the apparently safe 4.5 mg/day (Walravens et al., 1976) and unsafe 16 mg/day (Botash et al., 1992). No recent revisions have been made to the UL for this age group by any expert group, although any revision is likely to increase the UL and would therefore decrease the number of very young children in the current study identified as consuming a high zinc intake.

Several food components in the diet can affect zinc bioavailability, in particular phytate which is a powerful inhibitor of zinc absorption, at least in adults (Institute of Medicine, 2002). Although there was a tendency for there to be a higher phytate-to-zinc molar ratio in BLISS compared with Control infants at 7 months ($p=0.06$), this is unlikely to be of concern as the median phytate-to-zinc molar ratios were considerably lower than 15 for both groups at 7 and 12 months. Such values indicate that the average diets of both groups are likely high in bioavailability, as only ratios above 15 have been shown to have detrimental effects on the bioavailability of zinc (World Health Organization, 1996), although it is not known whether these absorption estimates are applicable to infants (Gibson et al., 2010). It is important to note that there is currently some controversy over whether dietary phytate inhibits zinc absorption in infants and children as it does in adults (Miller et al., 2015), although no studies have yet specifically investigated the impact of different concentrations of phytate on zinc absorption in healthy infants.

Significant differences were observed in the food sources of zinc between groups in our study, despite no differences in overall energy or zinc intakes. Little comparable literature exists examining the contribution of complementary foods to the zinc intakes of infants under the age of 12 months from high-income countries. Previous studies have indicated that infants and toddlers (<1-3 years) receive the majority of their zinc intake from complementary foods from milk/dairy products (Arsenault et al., 2003; Michaelsen et al., 1994; Rangan et al., 2012; Fox et al., 2006b). The current findings support these previous studies as ‘dairy’ was one of the major contributors to zinc intakes from complementary foods at 7 months along with ‘vegetables’ in the Control group and ‘breads and cereals’ in the BLISS group, and was the predominant source of zinc intakes from complementary foods at
12 months in both groups. Although ‘dairy’ is high in calcium it is unlikely that this would have impaired zinc absorption as high calcium intakes have only been found to impact on zinc absorption when intakes of phytate are also high, which is not the case in this study.

Very little research has been conducted in high-income countries on zinc intakes and status during the complementary feeding period, despite concern that inadequate zinc status may have detrimental effects on the risk of infection (Shankar et al., 1998; Fraker et al., 2000), and growth (Nissensohn et al., 2016). This study, therefore, makes a useful contribution not just to our understanding of the impact of a baby-led approach to complementary feeding on zinc intakes and status, but also to our limited knowledge of zinc intakes and status during complementary feeding in general.

More than half of the infants in the current study had low plasma zinc concentrations, despite a very low prevalence of inadequate zinc intakes. Such disparity between indices of intake and biochemical status has been observed previously (Morgan et al., 2010; Krebs et al., 2006), raising questions about whether the current criteria for determining risk of zinc deficiency need to be adjusted for this age group. For infants 12 months of age, the current reference criteria for low biochemical zinc status is the same as for any child (male or female) under 10 years of age (Hotz et al., 2003; Brown et al., 2004), which is perhaps inappropriate due to the non-specific age range and variability in the physiological state (in particular growth rate) between birth and 10 years of age. Indices of zinc related functioning, such as growth, might clarify this issue. Studies in high-income countries have reported poorer growth in children with low zinc status (Gibson et al., 1989; Gibson et al., 2010). However, there was no evidence of growth faltering in our study participants (Taylor et al., 2017). We did not directly measure any other functional outcomes that may be associated with poorer zinc related function, so we do not have further information that would help determine whether zinc status was inadequate for optimal function in this group of toddlers.

Inflammation and infection have been shown to decrease plasma zinc concentrations in infants (Brown et al., 1993; Arsenault et al., 2011). It is
unlikely that this is the cause of the high prevalence of zinc deficiency in the current study, because blood collection was delayed in infants who were unwell until they had recovered, in order to minimise the influence of inflammation on plasma zinc. As a result, very few participants had signs of acute and chronic inflammation ($n=0$ with raised CRP alone; $n=2$ Control and $n=0$ BLISS with raised CRP and raised AGP; $n=6$ Control and $n=11$ BLISS with raised AGP alone).

It is also possible that a nutrient-zinc interaction could be contributing to the low plasma zinc concentrations seen in this study. It is well known that New Zealand soils are low in selenium (Lyons et al., 2004), so that New Zealand toddlers have low selenium status (McLachlan et al., 2004). The selenium-zinc interaction is at a cellular level, where selenium plays a role in regulating the concentration of zinc in the cells (Maret, 2000). This means that low selenium concentrations may prevent the delivery of zinc to the cells and reduce plasma zinc concentrations (Lyons et al., 2004). For this reason, it would be useful to determine whether zinc status is associated with selenium status in these toddlers (see Chapter 6).

The current study has a number of strengths: first, that it is the first study internationally to examine whether a baby-led approach to complementary feeding is likely to have any impact on the biochemical zinc status of infants. Second, rigorous weighed food record data were collected using three non-consecutive days of weighed diet records. As infants often do not eat all of the food offered to them we asked parents to weigh the food before and after eating (including food that was no longer on the surface on which it was originally offered) to ensure we had an accurate representation of what was actually consumed by the infants. Recipes were also disaggregated so that ingredients in recipes could be assigned to individual food groups rather than being reported as ‘mixed dishes’. This prevented over- or under-estimation of foods consumed by infants within recipes. Third, strict trace element-free methods were used to collect and separate blood samples for determining plasma zinc concentrations, as recommended by IZiNCG (Brown et al., 2004).

The limitations of this study include that it was a secondary outcome of the BLISS study which was not specifically powered to investigate biochemical
zinc status. This means that the results should be interpreted with caution. However, the confidence intervals for the differences reported do provide the range of plausible differences between the groups, which are fairly narrow for both dietary zinc and plasma zinc. This suggests that even a larger study would be unlikely to identify large differences between the groups. Finally, the results cannot be generalised to infants following BLW, as this study investigated the impact of a modified version of BLW.

In summary, zinc intakes and biochemical zinc status appear to be similar in infants following a modified version of Baby-Led Weaning (BLISS) to those of infants following more traditional spoon-feeding, although the food sources of zinc are quite different. These results, however, cannot be generalised to infants following BLW; the intervention was specifically modified in the attempt to increase iron intake, and this may have improved zinc intakes. In fact, it is likely that BLISS did improve iron and zinc intakes since the only other study assessing dietary intakes of infants following a baby-led approach reported that infants following unmodified BLW had significantly lower iron and zinc intakes than infants who were traditionally spoon-fed (Morison et al., 2016). Although zinc intakes appeared to be adequate in both groups, the proportion of infants with low plasma zinc was high, supporting the call to reexamine the current criteria for determining inadequate zinc intake and biochemical zinc deficiency in this age group.
Potential predictors of zinc status in toddlers: a cross-sectional analysis

6-month-old Quinn decides which food to choose from: red meat (a food high in bioavailable zinc), avocado (a low zinc food), broccoli (a food with less bioavailable zinc)
6.1 Introduction

During very early childhood the risk of zinc deficiency is increased (Gibson et al., 2011), mostly because there is a higher physiological requirement for zinc due to the high growth rate during this time (Brown et al., 2004), but also because the typical complementary foods offered (e.g., fruit, vegetables, cereals) are generally low in absorbable zinc and after 6 months of age breast milk no longer provides sufficient zinc to meet requirements (World Health Organization, 2004b).

Suboptimal zinc status has been reported in very young children in New Zealand, although very few studies have assessed this (Morgan et al., 2010; Ferguson et al., 2004). Inadequate zinc status during very early childhood is associated with an increased risk of infection (Shankar et al., 1998; Fraker et al., 2000). This is particularly important as many toddlers participate in child care programmes where the exposure to illness is high (Cross et al., 2009). Inadequate zinc status can also have detrimental effects on growth (Nissensohn et al., 2016). Therefore, it is important to determine what factors may be modifiable during very early childhood that could improve the zinc status of this age group.

Several international studies have reported factors affecting biochemical zinc concentrations in very young children, however, these studies are often restricted to assessing methodological and biological factors (Brown et al., 1993; Strand et al., 2004; Arsenault et al., 2011; Engle-Stone et al., 2014; Galetti et al., 2016). To the Candidate’s knowledge, no study has assessed modifiable ‘predictors’ of zinc status in healthy infants or toddlers after standardising factors known to affect biochemical zinc concentrations (i.e. fasting status, time of the day of sample collection, season, acute phase protein status, and age).

The objective addressed in this chapter was to examine associations between biochemical, dietary, and other variables, and plasma zinc concentration, and to determine potentially modifiable ‘predictors’ of zinc status at 12 months of age.
6.2 Methods

6.2.1 Data collection
In this chapter, the intervention and Control groups of the BLISS study were combined in a cross-sectional analysis. Plasma zinc samples were collected at 12 months of age using trace-element free techniques and standardised procedures as recommended by the International Zinc Nutrition Consultative Group (IZiNCG) (IZiNCG, 2007). Strict procedures were used to control for the known predictors of plasma zinc concentrations: time of blood collection, fasting status, and inflammation (see Chapter 3, Section 3.10). Dietary data used in this chapter were from participants who provided a weighed three-day diet record (WDR) at 12 months of age. Although dietary intake was also assessed at 7 months of age, these data were not used as plasma zinc is a relatively short term measure of zinc status (IZiNCG, 2007), and therefore the use of 7 month dietary data was not considered to be relevant to zinc status collected at 12 months of age. All other relevant data collection methods are described in the Methods chapter (Chapter 3).

6.2.2 Statistical analysis
This chapter describes a secondary analysis using data from the BLISS study. All analyses were conducted using Stata, version 14.2 (StataCorp LP, Texas, US).

The outcome variable was plasma zinc concentration (μmol/L). Therefore, only participants who provided biochemical zinc data at 12 months of age were included in the analyses (n=115). Due to skewed data, dietary variables are presented as medians and lower and upper percentiles (25th and 75th). All other participant characteristic data are presented as means and standard deviations.

The term ‘predictor’ is in inverted commas to acknowledge that in a cross-sectional analysis it is not possible to determine the direction of the association.
Univariate analyses were conducted for all ‘predictor’ variables (below) that were decided a priori to be of interest, either based on previous associations described in the literature, or because they were considered to potentially have an association with zinc status in toddlers (Chapter 2, Section 2.7.3). The variables from baseline that were investigated were: sex, parity, maternal education, and socioeconomic status (SES) – assessed as the level of household deprivation (New Zealand Index of deprivation (NZDep) score) (Atkinson et al., 2014). The variables from 12 months that were investigated were: plasma selenium concentration, haemoglobin concentration, food fussiness, length-for-age z-score, weight-for-age z-score, topical zinc preparation use in the past month; and the intake of: total dietary zinc, red meat, phytate, estimated breast milk (see Chapter 3, Section 3.9.1), measured infant formula (all formulas were iron and zinc fortified), cow’s milk, dairy (excluding cow’s milk), and ‘meat, fish, poultry’. The age complementary foods were introduced was also investigated. Although other variables are also known to predict zinc status in very early childhood (e.g., age, season, time of blood collection, fasting status, and inflammation), these factors were standardised during data collection and therefore not included in the analyses (their values are reported in Appendix S).

Univariate unadjusted and adjusted (for group) linear regression analyses were used to describe associations between each ‘predictor’ variable and plasma zinc concentration individually.

A multivariable model was then constructed to determine the modifiable ‘predictors’ of plasma zinc concentration. Variables with a univariate adjusted association of \( p < 0.10 \), that were also potentially modifiable, were considered for inclusion in the final multivariate model. Haemoglobin concentration, maternal education, and breast milk intake were excluded, even though they met the \( p < 0.10 \) criterion because they were not considered to be modifiable. Although mothers who were breastfeeding at 12 months could potentially modify the amount their infant was given, it was not considered to be practically possible to start breastfeeding at 12 months if the infant had been weaned. Group was
not included in the final multivariate model as it appeared to have very little impact on the correlation coefficients in the univariate analyses.

The multivariable model was constructed as follows: first, Pearson’s correlation was used to assess any potential issues with collinearity. This was considered to be particularly likely to occur between dietary variables. A correlation \((r)\) of \(\geq 0.7\) between two variables was considered to indicate that a collinear effect was likely if both variables were included in the final multivariate model (Dormann et al., 2013). Second, a stepwise linear bootstrap regression of 500 samples was used to select variables for inclusion with the highest consistency as a ‘predictor’. The variables which were included in more than half of the analyses (>250) were considered to be the strongest variables and were included in the final multivariable model. Prior to interpretation of the analysis, the residuals were plotted and assessed to be normal.
6.3 Results

6.3.1 Maternal and infant characteristics at baseline

A total of 115 blood samples were analysed for plasma zinc concentration. Although 119 blood samples were initially collected as part of the BLISS study, four samples had insufficient plasma to be able to assess plasma zinc concentration, as this was a secondary outcome to iron status.

Baseline maternal and infant characteristics are presented in Table 6.1 for participants who provided a biochemical zinc sample ($n=115$). Similar proportions of participants were primiparous and multiparous, 73% self-identified as New Zealand European ethnicity, and 52% of parent participants had a university qualification. The level of household deprivation was high for 35% of participants (similar to the 30% for the New Zealand population (Atkinson et al., 2014)). The proportions of female (53%) and male (47%) toddlers in the study were similar.
Table 6.1 Maternal and infant baseline characteristics of participants who provided biochemical zinc data at 12 months of age

<table>
<thead>
<tr>
<th>Maternal and household variables at baseline</th>
<th>Total (n=115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal parity</td>
<td></td>
</tr>
<tr>
<td>First child</td>
<td>44 (38)</td>
</tr>
<tr>
<td>Two children</td>
<td>47 (41)</td>
</tr>
<tr>
<td>Three or more children</td>
<td>24 (21)</td>
</tr>
<tr>
<td>Maternal ethnicity</td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>84 (73)</td>
</tr>
<tr>
<td>Māori</td>
<td>22 (19)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (8)</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
</tr>
<tr>
<td>School only</td>
<td>33 (29)</td>
</tr>
<tr>
<td>Post-secondary</td>
<td>22 (19)</td>
</tr>
<tr>
<td>University</td>
<td>60 (52)</td>
</tr>
<tr>
<td>Household deprivation</td>
<td></td>
</tr>
<tr>
<td>1-3 (Low)</td>
<td>30 (26)</td>
</tr>
<tr>
<td>4-6</td>
<td>45 (39)</td>
</tr>
<tr>
<td>7-10 (High)</td>
<td>40 (35)</td>
</tr>
<tr>
<td>Infant variables at baseline</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>61 (53)</td>
</tr>
<tr>
<td>Male</td>
<td>54 (47)</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>58 (50)</td>
</tr>
<tr>
<td>BLISS</td>
<td>57 (50)</td>
</tr>
</tbody>
</table>

*a*Data presented as n (%)  
*b*Other ethnicities were Asian and Pacific  
*c*Household deprivation categorised using the NZDep scale in which decile 1 indicates the lowest level of deprivation and 10 indicates the highest (Atkinson et al., 2014)  
*d*As part of the BLISS randomised controlled trial, participants were randomised to either the Control or BLISS group after stratification for maternal education and parity
6.3.2 Participant characteristics at 12 months of age

Mean characteristics of toddlers who provided biochemical zinc data at 12 months of age are presented in Table 6.2. The mean (SD) plasma zinc concentration was 9.7 (1.5) μmol/L, with 60% (n=69) of toddlers below the IZiNCG reference value of <9.9 μmol/L (IZiNCG, 2007) (Figure 6.1). Analyses for haemoglobin and selenium were available for 114 and 111 of participants with a plasma zinc concentration, respectively. Of the 115 toddlers who provided a biochemical sample for zinc analysis, 104 also provided dietary intake data at 12 months of age.

One (0.9%) participant was considered stunted (using the World Health Organization (WHO) classification of a length-for-age z-score <−2 SD (Gorstein et al., 1994)) and another (0.9%) was underweight (using the WHO classification of a weight-for-age z-score <−2 SD (Gorstein et al., 1994) at 12 months of age.

![Figure 6.1](image)

**Figure 6.1** Plasma zinc concentrations (μmol/L) of participants (n=115)
Table 6.2 Characteristics of participants who provided biochemical zinc data at 12 months of age

<table>
<thead>
<tr>
<th>Biochemical variables</th>
<th>n (%)</th>
<th>Mean (SD)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma zinc, μmol/L</td>
<td>115 (100)</td>
<td>9.7 (1.5)</td>
</tr>
<tr>
<td>Haemoglobin, g/L</td>
<td>114 (99)</td>
<td>117 (8.7)</td>
</tr>
<tr>
<td>Plasma selenium, μmol/L</td>
<td>111 (96)</td>
<td>0.83 (0.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary variables [median (25th, 75th percentile)]</th>
<th>n (%)</th>
<th>Mean (SD)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ/day</td>
<td>104 (90)</td>
<td>3,543 (3,090, 4,168)</td>
</tr>
<tr>
<td>Zinc, mg/day</td>
<td>104 (90)</td>
<td>4.4 (3.7, 5.4)</td>
</tr>
<tr>
<td>Phytate, mg/day</td>
<td>104 (90)</td>
<td>230 (150, 318)</td>
</tr>
<tr>
<td>Phytate:zinc molar ratiob</td>
<td>104 (90)</td>
<td>5.0 (3.4, 7.1)</td>
</tr>
<tr>
<td>Meat, fish, poultry, g/day</td>
<td>104 (90)</td>
<td>18.9 (9.5, 30.5)</td>
</tr>
<tr>
<td>Red meat, g/day</td>
<td>104 (90)</td>
<td>4.4 (0, 11.5)</td>
</tr>
<tr>
<td>Breast milk, g/day</td>
<td>104 (90)</td>
<td>201 (0, 448)</td>
</tr>
<tr>
<td>Infant formula, g/day</td>
<td>104 (90)</td>
<td>0 (0, 346)</td>
</tr>
<tr>
<td>Cow’s milk, g/day</td>
<td>104 (90)</td>
<td>21.6 (5.9, 132)</td>
</tr>
<tr>
<td>Dairyc, g/day</td>
<td>104 (90)</td>
<td>39.9 (9.5, 73.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other variables</th>
<th>n (%)</th>
<th>Mean (SD)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>114 (99)</td>
<td>9.8 (1.1)</td>
</tr>
<tr>
<td>Length, cm</td>
<td>114 (99)</td>
<td>75.8 (2.6)</td>
</tr>
<tr>
<td>Weight-for-age z-scored</td>
<td>114 (99)</td>
<td>0.37 (0.96)</td>
</tr>
<tr>
<td>Length-for-age z-scored</td>
<td>114 (99)</td>
<td>0.26 (0.93)</td>
</tr>
<tr>
<td>Food fussiness scoref</td>
<td>114 (99)</td>
<td>2.1 (0.6)</td>
</tr>
<tr>
<td>Age complementary foods introduced, weeks</td>
<td>115 (100)</td>
<td>23.5 (3.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Topical zinc preparation use in the past month</th>
<th>n (%)</th>
<th>Mean (SD)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>59 (51)</td>
<td>-</td>
</tr>
<tr>
<td>No</td>
<td>56 (49)</td>
<td>-</td>
</tr>
</tbody>
</table>

aData presented as mean (SD), unless otherwise specified
bCalculated as [phytate (mg) / 660] / [zinc (mg) / 65.4]
cExcludes cow’s milk
dWeight-for-age z-score calculated using the World Health Organization child growth standards reference data (de Onis et al., 2006)
eLength-for-age z-score calculated using the World Health Organization child growth standards reference data (de Onis et al., 2006)
fFood fussiness was determined by six questions in the 12 month questionnaire completed by parent participants, using the method by Wardle et al. (2001) – lowest score: 1.0 and highest score: 5.0
6.3.3 Univariate associations between ‘predictor’ variables and plasma zinc concentrations

Variables associated with plasma zinc concentration with a \( p \) value of <0.10 were: haemoglobin concentration, maternal education, food fussiness score, and intake of: energy, zinc, ‘meat, fish, poultry’, red meat, breast milk, and infant formula (formulas contained 3-6 mg zinc per 100 g), in both the unadjusted and adjusted univariate analyses (Table 6.3). The significant association found between plasma zinc concentration and maternal education was that toddlers of mothers with university education had an almost 1 \( \mu \text{mol/L} \) lower plasma zinc concentration than toddlers whose mothers had a school education only, after adjusting for group \( (p=0.022) \) (Table 6.3).
Table 6.3 Univariate associations between ‘predictor’ variables and plasma zinc concentration (μmol/L) at 12 months of age

<table>
<thead>
<tr>
<th>Biochemical variables</th>
<th>Change in plasma zinc concentration (μmol/L)</th>
<th>Unadjusted</th>
<th>Adjusted for group&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>β (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Haemoglobin, g/L</td>
<td>114 (99)</td>
<td>0.05 (0.01, 0.08)</td>
<td>0.005</td>
</tr>
<tr>
<td>Plasma selenium, μmol/L</td>
<td>111 (96)</td>
<td>0.01 (-0.01, 0.03)</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Dietary variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kJ/day</td>
<td>104 (90)</td>
<td>0.00 (-0.00, 0.00)</td>
<td>0.07</td>
</tr>
<tr>
<td>Zinc, mg/day</td>
<td>104 (90)</td>
<td>0.23 (0.07, 0.39)</td>
<td>0.005</td>
</tr>
<tr>
<td>Phytate, mg/day</td>
<td>104 (90)</td>
<td>0.00 (-0.00, 0.00)</td>
<td>0.71</td>
</tr>
<tr>
<td>Meat, fish, poultry, g/day</td>
<td>104 (90)</td>
<td>0.02 (0.00, 0.03)</td>
<td>0.014</td>
</tr>
<tr>
<td>Red meat, g/day</td>
<td>104 (90)</td>
<td>0.02 (0.00, 0.03)</td>
<td>0.015</td>
</tr>
<tr>
<td>Breast milk, g/day</td>
<td>104 (90)</td>
<td>-0.00 (-0.00, -0.00)</td>
<td>0.039</td>
</tr>
<tr>
<td>Infant formula, g/day</td>
<td>104 (90)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.007</td>
</tr>
<tr>
<td>Cow’s milk, g/day</td>
<td>104 (90)</td>
<td>0.00 (-0.00, 0.00)</td>
<td>0.65</td>
</tr>
<tr>
<td>Dairy&lt;sup&gt;b&lt;/sup&gt;, g/day</td>
<td>104 (90)</td>
<td>-0.00 (-0.01, 0.00)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Other variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First child</td>
<td>44 (38)</td>
<td>1.00 (reference)</td>
<td>-</td>
</tr>
<tr>
<td>Two children</td>
<td>47 (41)</td>
<td>-0.17 (-0.81, 0.47)</td>
<td>0.60</td>
</tr>
<tr>
<td>Three or more children</td>
<td>24 (21)</td>
<td>-0.41 (-1.19, 0.36)</td>
<td>0.29</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>School only</td>
<td>33 (29)</td>
<td>1.00 (reference)</td>
<td>-</td>
</tr>
<tr>
<td>Post-secondary</td>
<td>22 (19)</td>
<td>-0.33 (-1.15, 0.50)</td>
<td>0.43</td>
</tr>
<tr>
<td>University</td>
<td>60 (52)</td>
<td>-0.77 (-1.42, -0.12)</td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td>Household deprivation&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 (Low)</td>
<td>30 (26)</td>
<td>1.00 (reference)</td>
<td>-</td>
</tr>
<tr>
<td>4-6</td>
<td>45 (39)</td>
<td>0.11 (-0.60, 0.83)</td>
<td>0.76</td>
</tr>
<tr>
<td>7-10 (High)</td>
<td>40 (35)</td>
<td>0.52 (-0.22, 1.25)</td>
<td>0.17</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>54 (47)</td>
<td>1.00 (reference)</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>61 (53)</td>
<td>-0.21 (-0.78, 0.36)</td>
<td>0.47</td>
</tr>
<tr>
<td>Weight-for-age z-score&lt;sup&gt;d&lt;/sup&gt;</td>
<td>114 (99)</td>
<td>0.02 (-0.28, 0.32)</td>
<td>0.91</td>
</tr>
<tr>
<td>Length-for-age z-score&lt;sup&gt;e&lt;/sup&gt;</td>
<td>114 (99)</td>
<td>0.15 (-0.16, 0.45)</td>
<td>0.35</td>
</tr>
<tr>
<td>Food fussiness score&lt;sup&gt;f&lt;/sup&gt;</td>
<td>114 (99)</td>
<td>-0.43 (-0.89, 0.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>Age complementary foods introduced, weeks</td>
<td>115 (100)</td>
<td>0.03 (-0.05, 0.11)</td>
<td>0.39</td>
</tr>
<tr>
<td>Topical zinc preparation use in the past month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>59 (51)</td>
<td>1.00 (reference)</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>56 (49)</td>
<td>-0.07 (-0.64, 0.50)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Bold** indicates p < 0.10

<sup>a</sup> Regression coefficient (β) represents the change in biochemical zinc concentration (μmol/L) per unit change in the relevant ‘predictor’

<sup>b</sup> Excludes cow’s milk

<sup>c</sup> Household deprivation categorised using the NZDep scale in which decile 1 indicates the lowest level of deprivation and 10 indicates the highest (Atkinson et al., 2014)

<sup>d</sup> Weight-for-age z-score calculated based on the World Health Organization standards (de Onis et al., 2006)

<sup>e</sup> Length-for-age z-score calculated based on the World Health Organization standards (de Onis et al., 2006)

<sup>f</sup> Food fussiness was determined by six questions in the 12 month questionnaire completed by parent participants, using the method by Wardle et al. (2001) – lowest score: 1.0 and highest score: 5.0
6.3.4 Multivariate regression analysis of ‘predictors’ of plasma zinc concentrations at 12 months of age

After excluding non-modifiable ‘predictor’ variables (haemoglobin concentration, maternal education, and breast milk intake), collinear relationships between independent variables were assessed. Potentially problematic collinearity was identified between energy and zinc intake ($r=0.82$), red meat and zinc intake ($r=0.73$), red meat and ‘meat, fish, poultry’ intake ($r=0.70$), and ‘meat, fish, poultry’ and zinc intake ($r=0.70$) (Table 6.4).

Table 6.4 Pearson’s correlation ($r$) analysis to identify potential collinearity between modifiable ‘predictor’ variables

<table>
<thead>
<tr>
<th>Dietary variables</th>
<th>Plasma zinc</th>
<th>Zinc</th>
<th>Energy</th>
<th>Red meat</th>
<th>Infant formula</th>
<th>MFP</th>
<th>Food fussiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma zinc</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>0.27*</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy</td>
<td>0.18</td>
<td>0.82**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red meat</td>
<td>0.24*</td>
<td>0.73**</td>
<td>0.59**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant formula</td>
<td>0.26*</td>
<td>0.38**</td>
<td>0.06</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFP</td>
<td>0.24*</td>
<td>0.70**</td>
<td>0.53**</td>
<td>0.70**</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food fussiness</td>
<td>-0.18</td>
<td>-0.07</td>
<td>-0.15</td>
<td>-0.01</td>
<td>0.10</td>
<td>0.01</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: MFP, ‘meat, fish, poultry’  
* $p<0.05$, ** $p<0.001$  
$^a$Presented as a correlation coefficient ($r$), where a coefficient of $>0.70$ (in bold) is considered to potentially pose problems with collinearity.
The six ‘predictor’ variables that remained after this step were: energy, food fussiness score; and intakes of infant formula, red meat, ‘meat, fish, poultry’, and zinc. Using stepwise linear bootstrap regression analysis, infant formula intake, food fussiness score, and red meat intake were considered to be the strongest modifiable ‘predictors’ of plasma zinc concentrations at 12 months of age, as they were included in more than half (>250) of the analyses (Table 6.5). These variables were used to construct the multivariate model.

**Table 6.5** Frequency of significant associations between ‘predictor’ variables and plasma zinc concentration in a stepwise bootstrap analysis (n=104)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency of variable selection (out of 500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant formula, g/day</td>
<td>391</td>
</tr>
<tr>
<td>Food fussiness score</td>
<td>361</td>
</tr>
<tr>
<td>Red meat, g/day</td>
<td>269</td>
</tr>
<tr>
<td>Meat, fish, poultry, g/day</td>
<td>129</td>
</tr>
<tr>
<td>Zinc, g/day</td>
<td>95</td>
</tr>
<tr>
<td>Energy, kJ/day</td>
<td>82</td>
</tr>
</tbody>
</table>

The multivariate regression analysis with plasma zinc concentration as the outcome demonstrates that toddlers had higher plasma zinc concentrations of 0.01 μmol/L per gram of red meat consumed per day ($p=0.028$), and 0.01 μmol/L per 10 grams of infant formula per day ($p=0.009$), respectively (Table 6.6). Food fussiness was also a significant ‘predictor’ of plasma zinc concentration. A one unit increase in food fussiness score (possible score range: 1.0 to 5.0, where the highest score represents increased food fussiness) was associated with a 0.54 μmol/L lower plasma zinc concentration ($p=0.021$).

The $R^2$ for the final model was 0.15 ($p=0.001$). This suggests that 15% of the variance in plasma zinc concentration (μmol/L) was explained by the ‘predictors’ in this model (red meat intake, infant formula intake, and food fussiness).
Table 6.6 Multivariate model of ‘predictors’ of plasma zinc concentrations at 12 months of age (n=103)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red meat intake, g/day</td>
<td>0.01 (0.002, 0.03)</td>
<td>0.028</td>
</tr>
<tr>
<td>Infant formula intake, 10 g/day</td>
<td>0.01 (0.02, 0.03)</td>
<td>0.009</td>
</tr>
<tr>
<td>Food fussiness score b</td>
<td>-0.54 (-0.99, -0.08)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

**Bold** indicates a statistically significant difference at p<0.05

aRegression coefficient (β) represents the change in biochemical zinc concentration (μmol/L) per unit change in the relevant ‘predictor’
bFood fussiness was determined by six questions in the 12 month questionnaire completed by parent participants, using the method by Wardle et al. (2001) – lowest score: 1.0 and highest score: 5.0
6.4 Discussion

In this cross-sectional analysis, several variables were statistically significantly associated with plasma zinc concentration in univariate analyses (i.e. haemoglobin, maternal education, and dietary intakes of: energy, zinc, ‘meat, fish, poultry’, red meat, breast milk, and infant formula). In contrast to findings from previous studies (Brown et al., 1993; Cavan et al., 1993; Ferguson et al., 2004; Engle-Stone et al., 2013; Houghton et al., 2016), no association was seen between plasma zinc concentration and plasma selenium, our measure of SES (level of household deprivation), growth indicators (length-for-age and weight-for-age z-scores), or dietary phytate intake. In the final full model, red meat intake, infant formula intake and food fussiness score were significant potentially modifiable ‘predictors’ of plasma zinc concentration at 12 months of age. The positive associations between intakes of red meat and infant formula and plasma zinc concentration were very small. In contrast, food fussiness score had a large inverse association with plasma zinc concentration. There has been no study to our knowledge that has determined modifiable ‘predictors’ of plasma zinc concentrations in healthy infants and toddlers.

Red meat intake, infant formula intake, and food fussiness score were significant independent ‘predictors’ of plasma zinc concentration at 12 months of age. Although the magnitudes of the coefficients appear to be very small for red meat and infant formula intakes (0.01 and 0.01 μmol/L, respectively), they may be of some clinical importance. An increased red meat intake of 20 grams per day$^1$ would increase plasma zinc concentration from the observed mean 9.7 μmol/L to the recommended reference level of 9.9 μmol/L. In total that would mean that red meat intakes would need to be on average 29 grams per day (mean red meat intake in both the BLISS and Control groups was ~9 grams per day, Chapter 4, Table 4.10). However, it is important to consider whether it is feasible for toddlers to consume this amount of red meat each day. A previous

$^1$ 9.9 μmol/L - 9.7 μmol/L = 0.2 μmol/L; coefficient = 0.01 μmol/L; therefore: 0.2/0.01 = 20 g/day.
intervention study which provided red meat dishes to toddlers (12-20 months) found low adherence to their red meat intervention, with toddlers consuming on average only 0.7 portions of the 2 portions (28 grams per portion) provided per day (Morgan et al., 2010). This would equate to 19.6 grams per day, on average 10 grams more than was consumed by participants in the current study, but 10 grams less than what would be required (29 grams - 9 grams = 10 grams) to increase plasma zinc concentrations to the recommended reference level. This suggests that modifying red meat intake alone is unlikely to be sufficient to increase plasma zinc concentrations meaningfully at 12 months of age.

For infant formula intake, an increased intake of 200 grams per day\(^2\) (just under 1 cup) would be required to increase plasma zinc concentration from the mean 9.7 μmol/L to the recommended reference level of 9.9 μmol/L (IZiNCG, 2007), for toddlers in this study. The average intake of infant formula would then increase from 329-414 grams per day (mean infant formula intake was 329 grams per day for BLISS and 414 grams per day for Control toddlers, Chapter 4, Table 4.10) to 529-614 grams per day. This amount of formula is still within the recommended fluid intakes for toddlers of 1000 mL or 4 cups (approximately 980 grams), but considerably higher than the up to 500 mL of milk recommended for this age (Ministry of Health, 2008). While this may seem achievable for toddlers, it is important to consider both the cost and other potential nutrient implications of consuming this amount of infant formula. The cost of infant formula could be a barrier for some families, particularly those of lower socio-economic status, and this higher intake of infant formula may displace solid foods in the diet which are important sources of nutrients for toddlers. It may also delay development of a more adult type diet.

\(^2\) 9.9 μmol/L - 9.7 μmol/L = 0.2 μmol/L; coefficient = 0.001 μmol/L; therefore: 0.2/0.001 = 200 g/day
Figure 6.2 Plausible pathways in the association between food fussiness and zinc status
A one-point higher **food fussiness** score was associated with a plasma zinc concentration that was 0.54 μmol/L lower in this group of toddlers. It is not possible to determine from this cross-sectional observational analysis whether food fussiness caused lower plasma zinc concentration (in which case intervening to improve food fussiness would improve biochemical zinc status; direction 1), or whether lower plasma zinc concentration caused higher food fussiness (in which case intervening to improve biochemical zinc status would improve food fussiness; direction 2) (Figure 6.2, A). In fact, there is a plausible cycle in which higher food fussiness would cause decreased zinc intakes, which would decrease zinc status, which would lead to impaired taste acuity, which in turn would lead back to higher food fussiness score (Figure 6.2, A). What we do not know is where the cycle starts in the current study, i.e. what the driver is.

Given the very limited previous literature in this area, it appears that increased food fussiness may result in decreased zinc intake, *pathway 1* (Figure 6.2, B). Carruth et al. (2004) reported that lower zinc intakes were found in infants (aged 9 to 11 months) who were classified as “picky” eaters, compared with “non-picky” eaters (Figure 6.2, B). The terms “picky” eating and food fussiness appear to refer to a similar phenomenon with both Carruth et al. (1998) and Wardle et al. (2001) describing it as being highly selective about the range, or the variety, of foods that are accepted. There is a plausible mechanism for this association - if an infant or toddler is more food fussy then perhaps their intake of foods high in zinc may be low. Moreover, they may consume fewer foods which have an enhancing effect on zinc absorption (e.g., red meat) so their absorption of zinc may also be lower.

In turn, decreased zinc intakes may result in poorer zinc status *pathway 2* (Figure 6.2, B). Although very few studies in very young children have reported a relationship between zinc intakes and zinc status (Salmenperä et al., 1994; Angelova et al., 2014), Hambidge et al. (1972) supplemented five zinc deficient children (aged 5-7 years; zinc deficiency identified using hair zinc) with 2 mg zinc per kg body weight for 1-3 months, after which hair zinc concentrations improved (Hambidge et al., 1972), suggesting an association between zinc intake and zinc status.
There is also evidence to suggest that lower zinc status results in impaired taste acuity (also known as hypogeusia), pathway 3 (Figure 6.2, B). In the same study reported above, Hambidge et al. (1972) found that taste acuity was impaired in the children who had low hair zinc concentrations, but that after these children were supplemented with zinc so that their hair zinc concentrations improved, taste acuity improved. Other studies confirm that poor taste acuity is a characteristic feature of poor zinc status (assessed using hair zinc concentration) in children (Buzina et al., 1980; Gibson et al., 1989; Cavan et al., 1993).

To close the cycle (pathway 4; Figure 6.2, B), impaired taste acuity may in turn result in higher food fussiness. Using retrospective diet histories, Hambidge et al. (1972) noted that the variety of foods consumed by zinc deficient children was limited. After supplementation, the improvement in taste acuity reported above was accompanied by an increase in food variety (Hambidge et al., 1972), suggesting an association between an improvement in taste acuity and increased variety of foods consumed.

It is not clear what the driver for the results of the current study is - whether higher food fussiness led to poorer zinc status, or whether poorer zinc status caused increased food fussiness - because the analysis is cross-sectional. Similarly, the study by Carruth et al. (2004) demonstrating an association between ‘picky’ eating and lower zinc intakes was also observational. While the study by Hambidge et al. (1972) appears to provide a plausible directional link for many of these pathways (2, 3, and 4; Figure 6.2, B), the study was limited by its lack of Control group, and use of hair samples rather than plasma samples for assessing zinc status. It was also conducted in children aged 5-7 years, so the results may not be applicable to toddlers.

The results of the current study are certainly worth exploring further to try and clarify the direction of causality, particularly given the magnitude of the association which suggests that: a decrease in food fussiness score of 1 point (167% of a SD) would result in an increase in plasma zinc concentration of 0.54 μmol/L (direction 1; Figure 6.2, A), or an increase in plasma zinc concentration of 0.54 μmol/L (36% of a SD) would result in a 1 point lower food fussiness.
score (direction 2; Figure 6.2, A). In fact, if the underlying pathway in this age group is driven by food fussiness, a change of 0.4 points in food fussiness score (2/3rds of a SD) would be expected to increase plasma zinc concentrations from the mean (9.7 μmol/L), to the recommended reference level of 9.9 μmol/L (IZiNCG, 2007). The only way to resolve the uncertainty about the direction of this association would be to conduct a randomised controlled trial of zinc supplementation, with measurement of changes in taste acuity and food fussiness; or an intervention to reduce food fussiness (such as the behavioural intervention by Birch et al. (1982) which reported an improvement in food preference of children by increasing the frequency of exposure to a food) with measurement of the subsequent impact on bioavailable zinc intake and plasma zinc concentration.

The final full multivariate model comprising red meat and infant formula intake, and food fussiness, explained 15% of the variance in plasma zinc concentrations. This highlights that many other factors are contributing to zinc status in toddlers that were not included in this final model. More work is necessary to determine further factors which are affecting plasma zinc concentrations in this age group, and whether any of these are also potentially modifiable.

In the initial steps of developing the final full model for the current study, variables were identified that were univariately associated with plasma zinc. These univariate analyses were conducted for 20 ‘predictor’ variables that had been chosen a priori either because of previous associations described in the literature, or because there was a plausible mechanism for an association with zinc status in toddlers (Chapter 2, Section 2.7.3). It is somewhat surprising that several of these variables (SES, length-for-age z-score, weight-for-age z-scores, phytate intake, zinc intake, timing of the introduction of complementary foods, dairy and cow’s milk intake, and plasma selenium) were not found to be associated with zinc status in the current study.

Several studies in very young children and school age children have reported an association between zinc status and SES, despite different variables being used to assess SES (Villalpando et al., 2003; Thurlow et al., 2005;
Arsenault et al., 2011; Engle-Stone et al., 2013; Galetti et al., 2016). Our finding is in contrast with these previous studies, as we found no association between plasma zinc concentration and SES (assessed by the level of household deprivation). However, our finding is comparable to that of another study in New Zealand European and Other (NZEO) children (aged 5-15 years) who also used the same index of deprivation score to assess household deprivation and did not see an association (Gibson et al., 2010). Perhaps the association is with some aspect of SES that is not captured by the NZDep index of deprivation score, for example maternal education. Interestingly, toddlers had lower plasma zinc concentrations if their mothers were university educated when compared with mothers with school only education. This finding could be because these toddlers were more likely to be breastfed if their mothers were more highly educated. We did see an inverse association between breast milk intake and zinc status in the univariate analysis, but the association between maternal education and breast milk intakes is speculative as we did not assess this in the current study.

It has been reported that very young children with low z-scores for weight-for-age (underweight) and length-for-age (stunted) are more likely to be zinc deficient, as zinc deficiency appears to be growth limiting (Brown et al., 2004). In the current study, we found no association between plasma zinc concentration and indicators of growth (length-for-age and weight-for-age z-scores). Only one participant was classified as stunted and one participant was classified as underweight at 12 months of age, using the WHO classification of <-2 SD (Gorstein et al., 1994). Therefore, it is not entirely surprising that no association was found between zinc concentration and weight-for-age or length-for-age z-scores, as a very small proportion were considered at the lower end of the range for both of these z-scores. In comparison, a Peruvian study found 16% of the children were classified as stunted and reported that length-for-age z-score was positively associated with serum zinc concentrations, although this association did not remain after adjusting for inflammation (Brown et al., 1993). The mean plasma zinc concentration in the current study (9.7 μmol/L) was below the recommended reference level of 9.9 μmol/L, but this does not appear
to have had a growth limiting effect on the majority of the otherwise healthy toddlers in this study, although it may be too early to detect any clinical consequences from these low plasma zinc concentrations.

Because **phytate** is a potent inhibitor of zinc absorption, at least in adults (Lönnerdal, 2000; Brown et al., 2004), we would have expected there might be an association with plasma zinc concentration. However, we found no association. Interestingly, despite the role of phytate as a powerful inhibitor of zinc absorption, just one study in Guatemalan children has found an association between high phytate intake and low hair zinc concentration (Cavan et al., 1993). It is important to note that no studies have assessed this relationship with plasma zinc concentration, nor assessed whether there is a relationship with hair or plasma zinc concentration in very young children.

In contrast to several studies (Michaelsen et al., 1994; Kattelmann et al., 2001; Taylor et al., 2004; Han et al., 2011; Cantoral et al., 2015), **zinc intake** was positively associated with plasma zinc concentration in the univariate association, however the association was not strong enough after the stepwise bootstrap analysis for inclusion in the final model.

It is also of interest that we found no association between plasma zinc concentration and the age when **complementary foods** were introduced. However, this could be because plasma zinc is only reflective of zinc intakes in the few weeks or months before collection (IZiNCG, 2007) and therefore would not reflect the period around 6 months earlier when complementary foods were introduced. If biochemical samples had been collected at 7 months of age, this would have given us a better understanding of the effect of introducing complementary foods on biochemical zinc status.

**Dairy** and **cow’s milk** are both relatively good sources of zinc intake, compared to fruits and vegetables (Ministry of Health, 2010), and toddlers in particular tend to consume large amounts of cow’s milk (Szymlek-Gay et al., 2010). We collected dietary data on the cusp of the age when it is recommended that very young children begin to consume cow’s milk (12 months of age) (Ministry of Health, 2008). It is likely the intakes of unmodified cow’s milk and dairy were still small for many of these 12 month olds.
Two studies in children (aged between 5-15 years) have shown strong correlations between selenium concentrations and zinc status (Krittaphol et al., 2006; Gibson et al., 2010). Gibson et al. (2010) found that serum selenium concentrations predicted plasma zinc concentrations in children self-reporting as NZEO ethnicity, or Pacific ethnicity, but not those of Māori ethnicity. We found no association between plasma selenium and zinc concentrations in the current study. However, we did not have the sample size to be able to assess differences by ethnicity in our study, which could be a confounder in the relationship between selenium and zinc.

This study has a number of strengths, many of which have been previously mentioned in Chapters 4 and 5. Rigorous dietary data were collected using weighed diet records, a method which is recommended for use in estimating dietary intakes of very young children (Burrows et al., 2010). Proof of this quality dietary assessment data is well demonstrated in our results. Particularly notable was our ability to detect a significant association between dietary zinc intake and zinc status, albeit only in the univariate associations. We used strict trace element-free methods to collect and separate blood samples for determining plasma zinc concentrations, as recommended by IZiNCG (Brown et al., 2004). Also, we were able to minimise any effect of fasting status, and inflammation/infection on plasma zinc concentrations by using a rigorous presample protocol that included encouraging parents to feed their child milk 90 minutes before the blood test and then no other food or drink before the blood sample was collected, and delayed the blood test for 14 days if the child was unwell. Lastly, the questions used for assessing food fussiness came from the Children’s Eating Behaviour Questionnaire which was developed for, and validated, in children (Wardle et al., 2001).

It is important to note the limitations of the current study which include that this was a cross-sectional secondary analysis using data from the BLISS study – a randomised controlled trial that was not specifically designed to determine ‘predictors’ of zinc status. Biochemical samples were collected at 12 months of age, but the dietary data were collected between 12-14 months of age, as participants were assigned to record three non-consecutive days over a
three-week period. As plasma zinc concentration reflects relatively short term dietary intake (IZiNCG, 2007), the intakes presented here may not be a true reflection of dietary intakes before the blood sample was collected. This is particularly important at least for the participants who were in the Control group because intakes are likely to vary significantly at around 12 months of age when there is a gradual transition from consuming chopped foods to more family foods (Ministry of Health, 2008). Also, because stepwise bootstrap regression was used to determine variables for inclusion in the final multivariate model, this may have given an over-optimistic impression of ‘predictors’ of zinc status in this group (Altman et al., 1989), and conclusions from this study should be treated with caution. Lastly, as this was an observational study, causation, and the direction of associations, cannot be determined.

In summary, in this cross-sectional analysis, intakes of both red meat and infant formula were positively associated with plasma zinc concentrations, whereas food fussiness score was inversely associated with zinc status. Although higher intakes of both red meat and infant formula are potentially achievable in the diets of toddlers, it is important to consider the potential barriers associated with increasing intakes of both of these foods, particularly cost, and parental and toddler willingness to modify behaviour. This analysis provides good, and exciting, evidence for an association between food fussiness and zinc status, however, further studies are required to confirm the direction of this association.
7 Conclusions and recommendations

7.1 Summary and conclusions

The overall aim of this thesis was to determine whether infants following the BLISS approach to complementary feeding, a version of BLW modified to prevent iron deficiency, have an increased risk of iron, or zinc, deficiency compared with infants following traditional spoon-feeding. To date, little evidence exists regarding the iron and zinc intakes of infants following a baby-led approach to complementary feeding (Morison et al., 2016), and no studies have assessed the biochemical status of very young children to determine whether the proposed increased risk of iron deficiency actually occurs (Cameron et al., 2012b). Therefore, the results presented in this thesis provide an important contribution to research examining the advantages and disadvantages of baby-led approaches to complementary feeding, particularly as this was the first randomised controlled trial conducted.

The results indicate that a baby-led approach to complementary feeding (BLISS) does not appear to increase the risk of iron or zinc deficiency when parents are given advice to offer ‘high-iron’ foods such as red meat and iron-fortified infant cereal at every meal. The BLISS intervention was specifically modified in an attempt to increase iron intakes, but because iron and zinc are found in similar foods, such modifications would be expected to influence zinc intakes as well.

There were no significant differences observed in the iron and zinc intakes of infants and toddlers following the BLISS approach to complementary feeding and those of infants and toddlers following traditional spoon-feeding. However, it is likely that the BLISS approach did improve iron and zinc intakes relative to those following unmodified BLW, as the only other study which has
examined nutrient intakes of infants following a baby-led approach to complementary feeding showed significantly lower intakes of iron and zinc amongst infants following BLW (Morison et al., 2016).

While there was no evidence of differences in iron and zinc intakes and status between the two approaches to complementary feeding, it is important to note the discrepancy between the dietary and biochemical findings of both iron and zinc observed in this study. There appeared to be a high proportion of infants (7 months of age) at risk of inadequate iron intakes, however, the majority of toddlers (12 months of age) had sufficient iron status. The inverse was observed for zinc, with seemingly adequate zinc intakes (at 7 and 12 months of age) but a high prevalence of zinc deficiency in toddlers (12 months of age). These discrepancies have been seen in other studies in very young children (Morgan et al., 2010; Soh et al., 2001; Soh et al., 2004; Krebs et al., 2006), and is likely due to the cut offs used for determining adequate intakes and status, which are often set using very little available data.

Red meat intake, infant formula intake and food fussiness were significantly associated with plasma zinc concentration in toddlers and may be important modifiable ‘predictors’ of zinc status. Although higher intakes of red meat and infant formula are potentially achievable in the diets of toddlers, it is important to consider the potential barriers associated with increasing intakes of both of these foods, particularly cost, and parental and toddler willingness to modify behaviour. Food fussiness was significantly associated with zinc status and may be a strong ‘predictor’ of zinc status in toddlers, however, the direction of this relationship needs to be determined.

### 7.2 Recommendations for future research

The findings in this thesis illustrate that iron and zinc remain as nutrients of concern in this age group, and work is still required to improve the iron and zinc intakes and status of very young children, regardless of the method of complementary feeding.
Given the discrepancy between the dietary and biochemical findings in this thesis and other studies (Morgan et al., 2010; Soh et al., 2001; Soh et al., 2004; Krebs et al., 2006), further work is needed to clarify the cut offs for determining adequate intakes and sufficient status in these age groups. Of particular importance is the cut off for determining zinc deficiency in very young children. Currently, the cut off is the same as for any child under 10 years of age, which is perhaps inappropriate due to marked variability in the growth rate between birth and 10 years of age. Although the percentage of infants with insufficient iron status appears similar to previous literature, a different method, based on body iron, was used for determining the stages of iron deficiency. Body iron was used because it has been shown to be less affected by inflammation and gives an indication of the stage of iron deficiency between depleted iron and iron deficiency anaemia, therefore, providing a continuum of the stages of iron deficiency (Cogswell et al., 2009). However, whether body iron is an appropriate measure for use in very young children is yet to be determined.

Further research is required to determine whether a more intensive intervention could improve iron intakes in very young children by increasing the amount of ‘high-iron’ foods (such as red meat and iron-fortified infant cereal) consumed, irrespective of whether the infant is following BLISS or traditional spoon-feeding. Due to concerns regarding infant health, it would be important to determine the inorganic arsenic content of infant rice cereals available for purchase in New Zealand, and to ensure that intakes remain below the WHO benchmark dose limits for inorganic arsenic intake of 3.0 μg/kg body weight per day (World Health Organization, 2010).

Determining the extent to which the zinc status of toddlers can be modified by red meat intake, infant formula intake, and food fussiness is recommended in future studies. However, the direction of the relationship between food fussiness and zinc status first needs to be confirmed by either conducting a randomised controlled trial of zinc supplementation, with measurement of changes in taste acuity and food fussiness; or an intervention to reduce food fussiness (such as the behavioural intervention by Birch et al. (1982) which reported an improvement in food preference of children by
increasing the frequency of exposure to a food) with measurement of the subsequent impact on bioavailable zinc intake and plasma zinc concentration.

As BLISS was the first randomised controlled trial globally to assess a baby-led approach to complementary feeding, further work could be undertaken to determine if this approach is feasible amongst different demographic and ethnically diverse populations, either in a more representative sample within New Zealand, or in another country where baby-led approaches are also popular.

7.3 Recommendations for clinical use

Before any recommendations can be made for clinical use, it is most important to note that the BLISS study assessed a modified version of BLW so no conclusions or recommendations can be made about the risk of iron and zinc deficiency in infants following unmodified BLW.

The New Zealand Ministry of Health currently does not support unmodified BLW due to the lack of research in this area (Ministry of Health, 2012). However, the popularity of unmodified BLW appears to be increasing amongst parents both in New Zealand (Cameron et al., 2013) and globally (Brown et al., 2011a; D’Andrea et al., 2016; Cichero, 2016; Beal, 2016). Therefore, if parents are choosing to follow a baby-led approach to complementary feeding regardless of the potential risks involved, the results of this thesis as well as the wider findings of the BLISS study could be used to help generate recommendations because this modified approach does not appear to result in any negative effects on iron and zinc status (results of this thesis), growth faltering (Taylor et al., 2017), or choking (Fangupo et al., 2016) in infants.

The practicality of the BLISS approach in the community setting needs to be determined, as the intervention involved a large amount of individualised time with both a lactation consultant and trained research staff to provide advice. Given that not all parents are interested in this alternative approach to complementary feeding (Cameron et al., 2013), it may not be appropriate to
educate Well Child programme providers to give such advice, as they already have a wide range of topics to cover in a limited time frame (Ministry of Health, 2014). Therefore, it may be more appropriate for this information to be provided just to parents who are interested in this approach, potentially as group education sessions presented by a healthcare professional, such as a dietitian.

Overall, this thesis provides intriguing results demonstrating no risk in terms of iron and zinc status for infants following a baby-led approach to complementary feeding, as long as parents are advised to offer ‘high-iron’ foods with each meal. However, it is important to highlight the need for further work to identify effective ways to achieve adequate iron and zinc intakes and status in this age group – no matter what method of complementary feeding is being used.
References


Nutrient Reference Values for Australia and New Zealand: Including Recommended Dietary Intakes, Canberra, Australia: NHRMC.


Southern Community Laboratories Ltd. 2014. *Southern Community Laboratories Paediatric Reference Ranges.* Southern Community Laboratories.


Appendices

Appendix A: BLISS baseline questionnaire
Appendix B: BLISS in a nutshell resource
Appendix C: BLISS laminated everyday foods to offer resources
Appendix D: Breastfeeding and solids questionnaire
Appendix E: 12-month questionnaire
Appendix F: BLISS weighed three-day diet record
Appendix G: Weighed diet record calculation sheet and entry protocol
Appendix H: Protocol for checking weighed diet records
Appendix I: Estimation of breast milk intakes at 12 months of age
Appendix J: Contribution of individual foods to food groups
Appendix K: Protocol for arranging blood collection appointment
Appendix L: Blood test appointment card
Appendix M: Pre-blood test instruction sheet
Appendix N: Protocol for reminder blood test phone call and illness questionnaire
Appendix O: Protocol for blood sample collection and analysis
Appendix P: Checklist for during blood collection appointment
Appendix Q: Zinc questionnaire for during blood collection appointment
Appendix R: Protocol for communicating abnormal blood results
Appendix S: Analysis of methodological factors known to affect plasma zinc concentrations
Appendix A: BLISS baseline questionnaire
Mother's Baseline Questionnaire

Welcome and thank you for being part of the BLISS study. This questionnaire is split into 2 sections and should take about 10 minutes to complete. Please answer every question - there are no right or wrong answers. Please ask the researchers if you have any questions - thank you for your time.

Section 1: Demographics

This section asks questions that will tell us how similar the people who are a part of BLISS are to other New Zealanders.

1 What is your date of birth? _____ / _____ / _______
   day   month   year

2 What is your expected date of delivery? _____ / _____ / _____
   day   month   year

3 How many weeks pregnant are you now? _____ weeks

4 Which ethnic group(s) do you belong to? Please tick all the boxes that apply
   ○ NZ European
   ○ Māori
   ○ Samoan
   ○ Tongan
   ○ Cook Island Māori
   ○ Niuean
   ○ Chinese
   ○ Indian
   ○ Other (such as Dutch, Japanese, Tokelauan). Please state: __________

5 Are you descended from a Māori (that is do you have a Māori birth parent, grandparent or great-grandparent etc)?
   ○ Yes
   ○ No Please go to question 7
   ○ Don’t know Please go to question 7

6 Do you know the name(s) of your Iwi (tribe)?
   ○ Yes If yes, please list your Iwi __________________________
   ○ No
7 Which ethnic group(s) does your baby's father belong to? Please tick all the boxes that apply

- NZ European
- Maori
- Samoan
- Tongan
- Cook Island Maori
- Niuean
- Chinese
- Indian
- Other (such as Dutch, Japanese, Tokelauan). Please state: _____________

8 What is your marital status?

- Single
- Married/Civil union
- Separated/Divorced/Widowed
- Partner/De facto
- Boyfriend/Girlfriend

9 What is your highest qualification? Don't count qualifications that take less than 3 months of full-time study to get

- Primary school
- NZ School Certificate in one or more subjects or National Certificate level 1 or NCEA level 1
- NZ Sixth Form Certificate in one or more subjects or National Certificate level 2 or NZ UE before 1986 in one or more subjects or NCEA level 2
- NZ Higher School Certificate or Higher Leaving Certificate or NZ University Bursary/Scholarship or National Certificate level 3 or NCEA level 3
- NZ trade certificate
- Polytechnic diploma or degree
- University undergraduate degree
- University postgraduate degree

10 How many people live in your household? Including yourself ____________
11 In addition to yourself, who else will your baby live with? Please tick all the boxes that apply

- Child’s father
- Your partner, but not child’s father
- Brothers or sisters (include step brothers/sisters)
- Child’s grandparents
- Other relatives
- Non-family members (e.g. boarder)
- No-one else besides you

12 Have you taken any of the following supplements during this pregnancy? Please tick all that apply and state the brand name.

- Elevit
- Vitamin D please state brand name: ________________
- Women’s pregnancy vitamin please state brand name: ________________
- Other please state type (e.g. Iron supplement): ________________
  please state brand name: ________________

Questions 13 to 16 ask about your situation when you became pregnant

13 Were you in paid employment?

- No, I was not in paid employment
- I was employed part-time (include self-employed)
- I was employed full-time (include self-employed)

14 Were you studying at University or Polytechnic?

- No, I was not studying
- I was a part-time student
- I was a full-time student

15 How tall were you without shoes? This is probably also your current height

_____ cm or _____ feet and _____ inches

16 How much did you weigh?

_____ kg or _____ stone and _____ pounds or _____ pounds

Questions 17 and 18 ask about your baby's biological father

17 How tall is he without shoes?

_____ cm or _____ feet and _____ inches
How much does he weigh?

______ kg  or  ______ stone and ______ pounds  or  ______ pounds

Section 2: Infant feeding

This section asks about how you plan to feed this baby, and if you have other children, how you fed them as babies.

19  Do you plan to breastfeed your child?

- Yes
- No  Please go to question 22

20  At what age do you plan to stop exclusively breastfeeding your child? The term exclusively breastfed means that the infant receives only breast milk and nothing else except medicine. Please give your answer as their age in days, weeks or months.

_________ days  or  _________ weeks  or  _________ months of age

- Don't know

21  At what age do you plan to stop all breastfeeding? Please give your answer as you infant’s age in days, weeks or months.

_________ days  or  _________ weeks  or  _________ months of age

- Don't know

22  At what age do you plan to introduce solid foods? Please give your answer as your infant’s age in days, weeks or months.

_________ days  or  _________ weeks  or  _________ months of age

- Don't know

Questions 23 to 25 are about starting your baby on solids.
23  At what age is it currently recommended that a child is first given solid foods? Please give your answer as the child’s age in days, weeks or months.

_________ days  or  __________ weeks  or  __________ months of age

☐ Don’t know

24  How do you plan to feed your baby when they first start eating solid foods?

☐ Spoon fed by adult
☐ Mostly spoon fed by adult, some baby feeding themselves
☐ About half spoon feeding by adult and half baby feeding themselves
☐ Mostly baby feeding themselves, some adult spoon feeding
☐ Baby feeding themselves
☐ Don’t know or not yet decided

25  What type of food do you plan to feed your baby when they first start eating solid foods?

☐ All puréed or mashed foods (including cans or jars of baby food, or food you purée yourself)
☐ Mostly puréed or mashed food, some finger foods
☐ About half puréed or mashed food and half finger foods
☐ Mostly finger foods and some puréed or mashed foods
☐ All finger foods (for example carrot sticks, broccoli floret, sliced toast)

26  Do you have other biological children?

☐ No this will be my first child  Please go to the end of the questionnaire
☐ Yes, 1 child
☐ Yes, 2 children
☐ Yes, 3 or more children

If you have more than one older child, please refer to the youngest child when answering questions 27 to 28.

27  How did you feed your youngest child when they first started eating solid foods?

☐ Spoon fed by adult
☐ Mostly spoon fed by adult, some baby feeding themselves
☐ About half spoon feeding by adult and half baby feeding themselves
☐ Mostly baby feeding themselves, some adult spoon feeding
☐ Baby feeding themselves
What type of food did you feed your youngest child when they first started eating solid foods?

- All puréed or mashed foods (including cans or jars of baby food, or food you purée yourself)
- Mostly puréed or mashed food, some finger foods
- About half puréed or mashed food and half finger foods
- Mostly finger foods and some puréed or mashed foods
- All finger foods (for example carrot sticks, broccoli floret, sliced toast)

Thank you for completing this questionnaire
Appendix B: BLISS in a nutshell resource
**BLISS in a nutshell**

1. **Start by offering foods that are adult finger-shaped.**
   Your baby will find food easier to grasp if it is a stick or finger size and shape. Pieces of food should be long enough so that your baby can hold it and there is still some of the food sticking out the top of their closed fist. Check the food is cooked enough by trying a piece yourself – you should be able to squash it on the roof of your mouth with your tongue – if you can’t then cook it a bit longer and test again.

2. **Always include your baby at meal times just as you would other family members.**
   Talk to them as they explore and eat their food and have some eye contact. Don’t feel you must talk to your baby about everything they eat or make eye contact all the time.

3. **Offer a variety of foods** from the resources including one energy rich food and one high iron food at each meal. Wherever possible, offer your baby the same foods that the rest of the family is eating, so that he feels part of what is going on. **Offer three or four different foods** at a meal (e.g. carrot, beef strip, cheese stick) and **start with one piece of each food.** You can always offer your baby “seconds”. Loading up your baby’s highchair with all sorts of foods will overwhelm him and there may be a lot of waste.

4. **Avoid hurrying your baby.**
   Allow her to decide the pace. In particular, don’t be tempted to “help” her by putting things in her mouth for her.

5. **Avoid offering ‘fast’ foods or foods that have added salt or sugar.**

6. **Always follow the basic safety rules**
   - Ensure that your baby is supported in an upright position – never leaning back – while he is eating. In the early days you can sit him on your lap, facing the table.
   - Don’t leave your baby alone with food. Ever!
   - **DON’T offer foods such as peanuts, popcorn, whole grapes or any food in a coin shape** – she may choke.

**DON’T Expect:**
- Your baby to eat any food on the first few occasions. Once he has discovered that these new toys taste nice, he will begin to suck, chew, and later, to swallow.
- A young baby to eat a whole piece of food at first as she won’t yet have developed the ability to get at food inside her fist.
- A young baby to have a perfectly “balanced” diet. There may be times when you think she is being a fussy or picky eater. Try to relax and allow your baby to explore foods, as they become more familiar with food and eating their acceptance of new tastes and texture will increase.

**Offer your baby food when she’s happy and content** – following a milk feed for babies until 8-9 months. In the early days it can be tricky coordinating a suitable time for the family meal and when your baby is awake and content. Don’t expect this to fall into place immediately but as you progress you’ll find a routine that suits all of you.
Appendix C: BLISS laminated everyday foods to offer resources
Everyday foods from 6 months of age

**High Iron Foods**
(Offer one of these foods at each meal)
- Strips of steak
- Apple slices
- Hummus
- Toast fingers (remove crusts) top with:
  - Baby rice cream cheese spread
  - Baby rice hummus spread
  - Pate
- Baby rice

**Energy Rich Foods**
(Offer one of these foods at each meal)
- Thick slice of avocado (not too ripe)
- Slices of cheese
- Toast fingers (remove crusts) top with:
  - Mashed baked beans
  - Mashed avocado or baby rice hummus spread
  - Margarine & smooth peanut butter
  - Margarine & cream cheese

**Easy Foods**
(See “First Foods Recipes” resource for cooking)
- Steamed or boiled:
  - Broccoli
  - Pumpkin
  - Carrots
  - Courgettes
  - Spinach
  - Sliced banana, soft peach, soft apricot, soft yellow kiwi/fruit
  - Soft mango
  - (Skin and stones removed)

Blue label = food also a Energy Rich food
Red label = food also a High Iron food
*Refer to “First Foods Recipes” resource for cooking and recipes

When your baby is sick offer some of the foods that have been circled (these are energy rich foods that are easy for your baby to eat) and remember to offer extra milk feeds. Your baby’s appetite may be reduced when they are unwell so also offer their favourite appetising foods.

---

**Foods to Offer Out and About or at Centre**

- Cheese sticks
- Hummus on toast
- Soft apricot
- Camembert wedges
- Power pikelets
- Baby yellow kiwi/fruit
- Soft peach
- Cooked pumpkin
- Banana
- Apple slices

Try taking a pot of hummus or baby rice to dip any of these foods into. You can also take toast fingers with the crusts cut off in a separate container.

*Refer to “First Foods Recipes” resource for cooking and recipes

Department of Human Nutrition, Department of Medicine and Department of Women’s and Children’s Health University of Otago P.O. Box 54, Dunedin Email: bliss@otago.ac.nz Phone: (03) 471 5683
Everyday foods to offer between 7 and 9 months of age

**Everyday Foods from 7-9 months**

- **High Iron Foods**
  - (Offer one of these foods at each meal)
  - Strips of steak
  - Lamb kofte
  - Lentil & white bean patties
  - High energy patties
  - Beef mince cakes
  - Tofu sticks
  - High iron banana biscuits
  - Baby rice cream cheese spread
  - Baby rice hummus spread
  - Baby rice
  - Hummus
  - Rusk

- **Energy Rich Foods**
  - (Offer one of these foods at each meal)
  - Thick slices of avocado (not too ripe)
  - Power pikelets
  - Sticks of cheese
  - French toast
  - Toast fingers (remove crusts)
  - Mashed avocado or baby ric
  - Avocado spread
  - Margarine & smooth peanut butter
  - Mashed beans
  - Mashed carrot
  - Margarine & cream cheese
  - Mashed banana

- **Easy Foods**
  - Steamed or boiled:
    - Broccoli
    - Green beans
    - Courgettes
    - Carrot
    - Pumpkin
  - Sliced banana, soft peach, soft apricot, soft yellow kiwifruit, strawberries, soft mango (skin and stones removed)

* Blue label = food also a Energy Rich food
* Red label = food also a High Iron food
* Refers to “First Foods Recipes” and “From 7 Months Recipes” resources for cooking and recipes

When your baby is sick offer some of the foods that have been circled (these are energy rich foods that are easy for your baby to eat) and remember to offer extra milk feeds. Your baby’s appetite may be reduced when they are unwell so also offer their favourite appetising foods.

---

**Foods to Offer Out and About or at Centre**

**From 7 months**

- Hummus on toast
- Soft peach camembert wedges
- Power pikelets
- Muffin
- Cheese & pineapple sandwiches
- Hard yellow kiwi/fruit
- Soft yellow kiwifruit
- Banana
- Apple crumb
- Egg sandwiches
- Smooth peanut butter sandwiches
- Cheese sticks
- Apple sticks
- Banana sticks

Try taking a potte of hummus or baby rice to dip any of these foods into. You can also take toast fingers with the crusts cut off in a separate container.

* Refer to “First Foods Recipes” and “From 7 Months Recipes” resources for cooking and recipes

---

Department of Human Nutrition, Department of Medicine and Department of Women’s and Children’s Health
University of Otago
PO Box 56, Dunedin
Email: bliss@otago.ac.nz Phone: (03) 476 5053

version 24.12

244
Appendix D: Breastfeeding and solids questionnaire
Breastfeeding and Solids - Feeding questionnaire

Welcome and thank you for being part of the BLISS study. This questionnaire should take about 5-10 minutes to complete.

Screening questions

1 Which of the following has your baby been fed in the last 48 hours?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Breast milk</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>b. Infant formula</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>c. Other liquids (not including minimal water)</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>d. Solid foods</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

2 Which of the following has your baby been fed at any time since birth, including in the hospital?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Breast milk</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>b. Infant formula</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>c. Other liquids (not including minimal water)</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>d. Solid foods</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

3 Consistency check (not visible on questionnaire)

Have warning alert if any of the following combinations appear:

If 1a = yes AND 2a = no [YES BM in last 48 hours AND NO BM since birth]
If 1b = yes AND 2b = no [YES IF in last 48 hours AND NO IF since birth]
If 1c = yes and 2c = no [YES OL in last 48 hours AND NO OL since birth]
If 1d = yes and 2d = no [YES Solids in last 48 hours AND NO solids since birth]

All other combinations okay

Answers to Q1 and Q2 determine skips according to following criteria:

If 2b = yes, go to Q4 [yes IF + any other answers=>Q4]
If 2c = yes, skip to Q5 [Yes OL + any other answers =>Q5]
If 2d = yes, skip to Q6a [Yes Solids =>Q6a]
If 2a = yes and 1a = no, skip to Q7 [Only BM BUT No BM in last 48 hours =>Q7]
If 2a = yes and 1a = yes, skip to Q11 [Only BM ever => Q11]
If $2a = \text{yes or no}$ and $2b = \text{yes and 2c = yes or no and 2d = yes or no}$, go to Q4

[Yes IF + any other answers => Q4]

If $2a = \text{yes or no}$ and $2b = \text{no and 2c = yes and 2d = yes or no}$, skip to Q5

[No IF + Yes OL + any other answers =>Q5]

If $2a = \text{yes or no}$ and $2b = \text{no and 2c = no and 2d = yes}$, skip to Q6a

[No IF + No OL + Yes Solids =>Q6a]

If $2b, 2c, 2d \text{ all = no and 2a = yes and 1a = no}$, skip to Q7

[Only BM BUT No BM in last 48 hours =>Q7]

If $2a = \text{yes and 2b, 2c, 2d all = no and 1a = yes}$, skip to Q11

[Only BM ever => Q11]

4 How old was your baby when they first had infant formula?

_______ days OR _______ weeks OR _______ months

If $2c = \text{yes}$, go to Q5

[Yes OL]

If $2d = \text{yes}$, skip to Q6a

[Yes Solids =>Q6a]

If $2a = \text{yes and 1a = no}$, skip to Q7

[Yes BM from birth BUT NO BM last 48 hours => Q7]

If $2b = \text{yes and 1b = no}$, skip to Q9

[Yes IF from birth BUT No IF last 48 hours => Q9]

If $2a = \text{yes and 1a = yes}$, skip to Q11

[Yes BM from birth + Yes BM last 48 hours => Q11]

If $2b = \text{yes and 1b = yes}$, skip to Q11

[Yes IF from birth + Yes IF last 48 hours => Q11]

If $2c = \text{no and 2d = yes}$, skip to Q6a

[No OL + Yes Solids =>Q6a]

If $2c = \text{no and 2d = no and 2a = yes and 1a = no}$, skip to Q7

[No OL or Solids + Yes BM from birth BUT NO BM last 48 hours => Q7]

If $2c = \text{no and 2d = no and 2b = yes and 1b = no}$, skip to Q9

[No OL or Solids + Yes IF from birth BUT No IF last 48 hours => Q9]

If $2c = \text{no and 2d = no and 2a = yes and 1a = yes}$, skip to Q11

[No OL or Solids + Yes BM from birth + Yes BM last 48 hours => Q11]

If $2c = \text{no and 2d = no and 2a = no and 2b = yes and 1b = yes}$, skip to Q11

[No OL or Solids or BM from birth + Yes IF from birth + No IF last 48 hours => Q9]
5. How old was your baby when they first had liquids other than breast milk or infant formula?

______ days  OR  _______ weeks  OR  _______ months

If 2d = yes, go to Q6a
If 2a = yes and 1a = no, skip to Q7
If 2b = yes and 1b = no, skip to Q9
If 2a = yes and 1a = yes, skip to Q11
If 2b = yes and 1b = yes, skip to Q11

If 2d = no and 2a = yes and 1a = no, skip to Q7
If 2d = no and 2b = yes and 1b = no, skip to Q9
If 2d = no and 2a = yes and 1a = yes, skip to Q11
If 2d = no and 2a = no and 2b = yes and 1b = yes, skip to Q11

6a. How old was your baby when they first started eating solid foods?

Note: “eating solid foods” means that baby appears to swallow at least some of the food.

______ weeks  OR  _______ months

6b. How was your baby fed when they first started eating solid foods?

Note: “Fed by adult” means that someone other than baby put the food in their mouth.
“Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.

○ Fed by adult
○ Mostly fed by adult, some baby fed themselves
○ About half fed by adult and half baby fed themselves
○ Mostly baby fed themselves, some fed by adult
○ Baby fed themselves
6c What type of foods did you give your baby when they first started eating solid food?

- All puréed or mashed foods (including cans or jars of baby food, or food you purée yourself, and dry foods that you add water to such as “baby rice” and porridge)
- Mostly puréed or mashed food, some finger foods
- About half puréed or mashed food and half finger foods
- Mostly finger foods and some puréed or mashed foods
- All finger foods (for example carrot sticks, broccoli floret, sliced toast)

If 1a = yes and 2b = yes and 1b = no, skip to Q9

[Yes BM last 48 hours + Yes IF since birth BUT No IF last 48 hours => Q9]

If 1a = yes and 2b = yes and 1b = yes, skip to Q11

[Yes BM last 48 hours + Yes IF since birth + yes IF last 48 hours => Q11]

If 2a = no and 2b = yes and 1b = no, skip to Q9

[No BM + Yes IF since birth BUT No IF last 48 hours => Q9]

If 2a = no and 2b = yes and 1b = yes, skip to Q11

[No BM + Yes IF since birth + Yes IF last 48 hours => Q11]

If 2a = no and 2b = no and 2d = yes, skip to Q12

[No BM + No IF + Yes Solids => Q12]

If 2a = no and 2b = no and 2d = no and 2c = yes, skip to Q31

[No BM + No IF + No Solids + Yes OL => Q31]

- If 2a = yes and 1a = yes and 2b = yes and 1b = no, skip to Q9
  [Yes BM +Yes BM last 48 hours + Yes IF since birth BUT No IF last 48 hours => Q9]

- If 2a = yes and 1a = yes and 2b = yes and 1b = yes, skip to Q11
  [Yes BM +Yes BM last 48 hours + Yes IF since birth + yes IF last 48 hours => Q11]

- If 2a = no and 2b = yes and 1b = no, skip to Q9
  [No BM + Yes IF since birth BUT No IF last 48 hours => Q9]

- If 2a = no and 2b = yes and 1b = yes, skip to Q11
  [No BM + Yes IF since birth +Yes IF last 48 hours => Q11]

- If 2a = no and 2b = no and 2d = yes, skip to Q12
  [No BM + No IF + Yes Solids => Q12]

- If 2a = no and 2b = no and 2d = no and 2c = yes, skip to Q31
  [No BM + No IF + No Solids + Yes OL => Q31]

7 Have you stopped breastfeeding?

- Yes
- No
• If $Q7 = no$ and $2b = yes$ and $1b = no$, Skip to $Q9$
  [No Stop BF + Yes IF BUT No IF last 48 hours => Q9]
• If $Q7 = no$ and $2b = yes$ and $1b = yes$, Skip to $Q11$
  [No Stop BF + Yes IF + Yes IF last 48 hours => Q11]
• If $Q7 = no$ and $2b = no$, skip to $Q11$
  [No Stop BF + No IF => Q11]

**8**  
How old was your baby when you stopped breastfeeding?  
_______ days OR _______ weeks OR _______ months

• If $2b = yes$ and $1b = yes$, skip to $Q11$
  [Yes Stop BF + Yes IF + Yes IF last 48 hours => Q11]
• If $2b = no$ and $2d = yes$, skip to $Q12$
  [Yes Solids => Q12]
• If $2b = no$ and $2d = no$ and $2c = yes$, skip to $Q31$
  [No IF + No Solids + Yes OL => Q31]

**9**  
Have you stopped feeding infant formula?

O Yes  
O No

• If $Q9 = no$, skip to $Q11$
  [No Stop IF => Q11]

**10**  
How old was your baby when you stopped feeding infant formula?  
_______ days OR _______ weeks OR _______ months

• If $Q9 = yes$ and $2a = no$ and $2d = yes$, skip to $Q12$
  [Yes Stop IF + No BF + Yes Solids => Q12]
• If $Q9 = yes$ and $2a = yes$ and $1a = no$ and $2d = yes$, skip to $Q12$
  [Yes Stop IF + Yes BF + No BF last 48 hours + Yes Solids => Q12]
• If $Q9 = yes$ and $1a = no$ and $1b = no$ and $2d = no$ and $2c = yes$, skip to $Q31$
  [Yes Stop IF + No BF last 48 hours + No Solids last 48 hours + Yes OL last 48 hours => Q31]
11 How has your baby been fed their milk (breast milk or infant formula) in the last **48 hours**?

*Note: Feeding on demand means feeding your baby as often as they want, day and night. Feeding on schedule means feeding your baby at set intervals.*

- Fed on demand
- Mostly fed on demand, some fed on schedule
- About half fed on demand and half fed on schedule
- Mostly fed on schedule, some fed on demand
- Fed on schedule
- Not fed breast milk or infant formula in the last 48 hours

- If $2d = no$, skip to Q31
  
  * [No Solids => Q31]

12 Does your baby feed **themselves** solids?

*Note: “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.*

- Yes
- No

- If Q12 = no, skip to Q20

13 How old was your baby when they first fed **themselves** solids?

*Note: “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.*

______ days  OR  ______ weeks

14 How was your baby fed when they first started feeding **themselves** solids?

*Note: “Fed by adult” means that someone other than baby put the food in their mouth. “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.*

- Mostly fed by adult, some baby fed themselves
- About half fed by adult and half baby fed themselves
- Mostly baby fed themselves, some fed by adult
- Baby fed themselves
15 Does your baby feed themselves solids regularly (at least once a day)?
*Note: Baby fed themselves means that baby picks up the food, puts it in their mouth and swallows some.*
- Yes
- No

- If Q15 = no, skip to Q20

16 How old was your baby when they started feeding themselves solids regularly (at least once a day)?
*Note: “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.*

_____ weeks OR _____ months

17 How was your baby fed when they first started feeding themselves solids regularly?
*Note: “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.*
- Mostly fed by adult, some baby fed themselves
- About half fed by adult and half baby fed themselves
- Mostly baby fed themselves, some fed by adult
- Baby fed themselves

18 Does your baby feed themselves all their food?  
(Excluding feeding by an adult when baby is feeling unwell).
*Note: “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.*
- Yes
- No

- If Q18 = no, skip to Q20

19 How old was your baby when they first started feeding themselves all their food?

_____ weeks OR _____ months
20 How has your baby been fed their solids in the last 48 hours?
Note: “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.

- Fed by adult
- Mostly fed by adult, some baby feeding themselves
- About half spoon feeding by adult and half baby feeding themselves
- Mostly baby feeding themselves, some adult feeding
- Baby feeding themselves

21 How has your baby been fed their solids in the past week?
Note: “Baby fed themselves” means that baby picks up the food, puts it in their mouth and appears to swallow at least some.

- Fed by adult
- Mostly fed by adult, some baby feeding themselves
- About half spoon feeding by adult and half baby feeding themselves
- Mostly baby feeding themselves, some adult feeding
- Baby feeding themselves

For questions 22-30, “eaten” means that food has been in baby’s mouth and s/he appears to swallow at least some.

22a Has your baby eaten Baby cereal?
- Yes
- No

- If Q22a = no, skip to Q23a

22b How old were they when they first ate Baby cereal?

\[ \text{\underline{\phantom{12345}}} \text{\phantom{12345}} \text{weeks} \quad \text{OR} \quad \text{\underline{\phantom{12345}}} \text{\phantom{12345}} \text{months} \]

23a Has your baby eaten Beef (includes mince, steak, sausages and roast beef)?
- Yes
- No

- If Q23a = no, skip to Q24a

23b How old were they when they first ate Beef?

\[ \text{\underline{\phantom{12345}}} \text{\phantom{12345}} \text{weeks} \quad \text{OR} \quad \text{\underline{\phantom{12345}}} \text{\phantom{12345}} \text{months} \]
**24a** Has your baby eaten **Lamb** (includes mince, steak, sausages and roast lamb)\
- Yes\
- No

• *If Q24a = no, skip to Q25a*

**24b** How old were they when they first ate **Lamb**?\
______ weeks  OR  _______ months

**25a** Has your baby eaten **Pork** (includes mince, ham, sausages and roast pork)?\
- Yes\
- No

• *If Q25a = no, skip to Q26a*

**25b** How old were they when they first ate **Pork** (includes ham)?\
______ weeks  OR  _______ months

**26a** Has your baby eaten **Chicken** (includes chicken pieces, mince, sausages and roast chicken)?\
- Yes\
- No

• *If Q26a = no, skip to Q27a*

**26b** How old were they when they first ate **Chicken**?\
______ weeks  OR  _______ months

**27a** Has your baby eaten **Fish**?\
- Yes\
- No

• *If Q27a = no, skip to Q28a*

**27b** How old were they when they first ate **Fish**?\
______ weeks  OR  _______ months

**28a** Has your baby eaten **Shellfish**?\
- Yes\
- No

• *If Q28a = no, skip to Q29a*

**28b** How old were they when they first ate **Shellfish**?
29a Has your baby eaten **processed meats** (luncheon sausage or Belgium)?
- Yes
- No

- **If Q29a = no, skip to Q30a**

29b How old were they when they first ate **processed meats**?

<table>
<thead>
<tr>
<th></th>
<th>weeks</th>
<th>OR</th>
<th>months</th>
</tr>
</thead>
</table>

30a Has your baby eaten **Beans, peas or lentils** (e.g. baked beans, hummus; NOT green peas or beans)?
- Yes
- No

- **If Q30a = no, skip to Q31**

30b How old were they when they first ate **Beans, peas or lentils** (e.g. baked beans, hummus; NOT green peas or beans)?

<table>
<thead>
<tr>
<th></th>
<th>weeks</th>
<th>OR</th>
<th>months</th>
</tr>
</thead>
</table>

31 How much of a problem is your baby’s sleeping pattern or habits for you?

<p>| | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

- No problem
- Small problem
- Moderate problem
- Large problem

32 In the last week what is the longest time your baby has slept in the night without waking?

<table>
<thead>
<tr>
<th></th>
<th>hours</th>
<th>OR</th>
<th>minutes</th>
</tr>
</thead>
</table>

33 Have you been worried about your baby’s weight gain since their birth?
- Yes, too much weight gain
- Yes, not enough weight gain
- No

**Thank you for completing this questionnaire**

256
Appendix E: 12-month questionnaire
12 month Questionnaire

Thank you for continuing to be part of the BLISS study. This questionnaire is split into 7 sections and should take about 40 minutes to complete. Please answer every question - there are no right or wrong answers. Please press the SAVE button each time that you see it. Please ask the researchers if you have any questions - thank you for your time.

Section 1: Feeding and health

1  Since you answered the “9 month Questionnaire”, has your baby had any illness that affected their feeding for more than 5 days?
   ○ No (please go to question 3)
   ○ Yes

   If yes, please describe the illness .................................................................

   ..........................................................................................................................

   If yes, please state how long it lasted ............. days .............. weeks

2  Were they hospitalised for this illness?
   ○ No
   ○ Yes

   If yes, for how many days? ............... 

3  In the past week, how often has your baby drunk all of his or her cup or bottle of milk?
   ○ Always
   ○ Most of the time
   ○ Sometimes
   ○ Rarely
   ○ Never
   ○ [Not applicable]

4  Has your baby gagged on food or drink in the past month?
   ○ No (please go to question 5)
   ○ Yes
If yes, how many times?  ............... per day    OR
.............. per week    OR
.............. per month

5 Has your baby choked on food or drink in the past month?

○ No (please go to question 10a)
○ Yes

If yes, how many times? ..............

6 Thinking of the most serious choking episode in the past month, which of the following did your baby do?
(Choose as many as apply)

○ Eyes watered
○ Pushed tongue out
○ Coughed
○ Gasped
○ Retched
○ Vomited
○ Cried
○ Went silent
○ Other Please state .................................................................

7 Thinking again of the most serious choking episode in the past month, which of the following happened?
(Choose as many as apply)

○ Baby resolved it themselves
○ Parent resolved it
○ A health professional resolved it
○ Another person resolved it
○ A health professional was involved
○ Baby was admitted to hospital
○ Other Please state .................................................................

8 Thinking again of the most serious choking episode in the past month, what was the food or drink responsible (please state whether it was raw or cooked)?

.................................................................
9 Thinking again of the most serious choking episode in the past month, what form was the food or drink in?

- Thin liquid
- Thick liquid
- Puréed
- Mashed
- Diced
- Sliced
- Whole

10 Thinking again of the most serious choking episode in the past month, who fed the baby the food or drink that was responsible?

- Baby him/herself
- Parent
- Another adult
- Brother or sister
- Another child

10a Has your baby choked on food or drink at any other time since your last measurement session with us (that is – not counting the past month)?

- No (please go to question 11)
- Yes

If yes, how many times? ............

10b Was the most serious choking incident since your last measurement visit (that is – including the past month) a choking incident that you described to us in questions 6-10 above?

- No
- Yes (please go to question 11)

10c Thinking of the most serious choking episode since your last measurement visit (but not including the past month), which of the following did your baby do? (Choose as many as apply)

- Eyes watered
- Pushed tongue out
- Coughed
- Gasped
- Rretched
○ Vomited  
○ Cried  
○ Went silent  
○ Other Please state …………………………………………………………………...

10d Thinking of the most serious choking episode since your last measurement visit (but not including the past month), which of the following happened? (Choose as many as apply)

○ Baby resolved it themselves  
○ Parent resolved it  
○ A health professional resolved it  
○ Another person resolved it  
○ A health professional was involved  
○ Baby was admitted to hospital  
○ Other Please state ………………………………………………………………………

10e Thinking of the most serious choking episode since your last measurement visit (but not including the past month), what was the food or drink responsible (please state whether it was raw or cooked)?

………………………………………………………………………………………………………………………

10f Thinking of the most serious choking episode since your last measurement visit (but not including the past month), what form was the food or drink in?

○ Thin liquid  
○ Thick liquid  
○ Puréed  
○ Mashed  
○ Diced  
○ Sliced  
○ Whole

10g Thinking of the most serious choking episode since your last measurement visit (but not including the past month), who fed the baby the food or drink that was responsible?

○ Baby him/herself  
○ Parent  
○ Another adult  
○ Brother or sister  
○ Another child
11 Has your baby eaten anything with wheat in it for the first time since you answered the 9 month questionnaire? (e.g., bread, toast, rusk, baby muesli, cake, biscuit, pikelet, flour)

圈  Yes
圈  No (please go to question 13)

12 How old were they when they first ate something with wheat in it?

______ months

Section 2: Parent experiences of feeding

13 Thinking about the way you are giving your baby solids, overall how messy is it?

1  2  3  4  5
Very messy  Not at all messy

14 Thinking about the way you are giving your baby solids, overall how convenient is it?

1  2  3  4  5
Very convenient  Very inconvenient

15 Thinking about the way you are giving your baby solids, overall how expensive is it?

1  2  3  4  5
Very expensive  Very inexpensive

16 Thinking about the way you are giving your baby solids, overall how happy are you with this way of giving solids?

1  2  3  4  5
Very happy  Very unhappy

17 As a parent, how frustrating do you find this way of giving your baby solids?

1  2  3  4  5
Very frustrating  Not at all frustrating
18. How well does this way of giving your baby solids suit you as a parent?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Suits me very well</td>
<td></td>
<td></td>
<td></td>
<td>Doesn't suit me at all</td>
</tr>
</tbody>
</table>

19. Where does your baby sit to eat their solids? (tick all that apply)

- Highchair
- Chair attached to table
- Baby sized chair on floor
- Floor
- Someone’s knee
- Other (Please state) .................................

20. How often do you, or another adult, sit with your child when they're eating?

- Never
- Occasionally
- About half the time
- Almost always
- Always

21. Overall, how acceptable do you find the amount of mess your baby makes when eating?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very acceptable</td>
<td>Neutral</td>
<td>Very unacceptable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

22. How uncomfortable do you feel about the amount of mess your baby makes when eating at home?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very uncomfortable</td>
<td>Neutral</td>
<td>Very comfortable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

23. How uncomfortable do you feel about the amount of mess your baby makes when eating away from home?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very uncomfortable</td>
<td>Neutral</td>
<td>Very comfortable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
24 Does your baby drop food on the floor?

- Yes, most meals
- Yes, some meals
- Rarely
- Never

25 Does your baby throw food around?

- Yes, most meals
- Yes, some meals
- Rarely
- Never

26 It takes a while for babies to learn to eat solids. How long did it take after your baby started solids before you felt that they were eating enough?

- less than a week
- 1 week
- 2 weeks
- 3 weeks
- 4 weeks
- 5 weeks
- 1½ months
- 2 months
- 2½ months
- 3 months
- more than 3 months
- they are not eating enough solids

27 Did your baby eat “finger foods” before they were 9 months of age? Finger foods are foods that your baby can pick up and feed themselves. (If your baby picks up a spoon and feeds themselves with it then include that as well).

- Yes (please go to question 30)
- No

28 Has your baby been offered any “finger foods” for the first time since they were 9 months of age?

- No (please go to question 32)
- Yes
29. How old was your baby when they first had finger foods (or fed themself with a spoon)?

- 9 months
- 9½ months
- 10 months
- 10½ months
- 11 months
- 11½ months
- 12 months
- 13 months
- 14 months
- Other

30. How long did it take after your baby started feeding themself finger foods before you felt that they were eating enough to only need finger foods? (Do not count drinks, including milk).

- less than a week
- 1 week
- 2 weeks
- 3 weeks
- 4 weeks
- 5 weeks
- 1½ months
- 2 months
- 2½ months
- 3 months
- more than 3 months
- they are not eating enough finger foods to only need finger food

31. How much of the food baby eats now is finger food they feed themself (or food that baby feeds themself with a spoon)? (Do not count drinks, including milk).

- None of it
- Some of it
- Most of it
- All of it

Section 3: Your child’s appetite

Please state whether you agree or disagree with each statement

32. My child knows how much food s/he should eat

- Disagree
- Slightly disagree
- Neutral
- Slightly agree
- Agree
33 My child stops eating when s/he is full

Disagree Slightly disagree Neutral Slightly agree Agree

34 My child knows when s/he should stop eating

Disagree Slightly disagree Neutral Slightly agree Agree

35 If my child is full s/he will not eat snacks

Disagree Slightly disagree Neutral Slightly agree Agree

36 My child eats even when s/he is not hungry

Disagree Slightly disagree Neutral Slightly agree Agree

37 If my child is full, s/he will not ask for more food

Disagree Slightly disagree Neutral Slightly agree Agree

38 My child knows when s/he is full

Disagree Slightly disagree Neutral Slightly agree Agree

39 My child eats even when s/he is already full

Disagree Slightly disagree Neutral Slightly agree Agree

Section 4: Your child and food

40 My child loves food

Never Rarely Sometimes Often Always
41. My child eats more when worried

Never  Rarely  Sometimes  Often  Always

42. My child has a big appetite

Never  Rarely  Sometimes  Often  Always

43. My child finishes his/her meal quickly

Never  Rarely  Sometimes  Often  Always

44. My child is interested in food

Never  Rarely  Sometimes  Often  Always

45. My child is always “asking” for a drink

Never  Rarely  Sometimes  Often  Always

46. My child refuses new foods at first

Never  Rarely  Sometimes  Often  Always

47. My child eats slowly

Never  Rarely  Sometimes  Often  Always

48. My child eats less when angry

Never  Rarely  Sometimes  Often  Always

49. My child enjoys tasting new foods

Never  Rarely  Sometimes  Often  Always
50  My child eats less when she/he is tired

Never  Rarely  Sometimes  Often  Always

51  My child is always “asking” for food

Never  Rarely  Sometimes  Often  Always

52  My child eats more when annoyed

Never  Rarely  Sometimes  Often  Always

53  If allowed to, my child would eat too much

Never  Rarely  Sometimes  Often  Always

54  My child eats more when anxious

Never  Rarely  Sometimes  Often  Always

55  My child enjoys a wide variety of foods

Never  Rarely  Sometimes  Often  Always

56  My child leaves food on his/her plate at the end of a meal

Never  Rarely  Sometimes  Often  Always

57  My child takes more than 30 minutes to finish a meal

Never  Rarely  Sometimes  Often  Always

58  Given the choice, my child would eat most of the time

Never  Rarely  Sometimes  Often  Always
<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>My child looks forward to mealtimes</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>60</td>
<td>My child gets full before his/her meal is finished</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>61</td>
<td>My child enjoys eating</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>62</td>
<td>My child eats more when she/he is happy</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>63</td>
<td>My child is difficult to please with meals</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>64</td>
<td>My child eats less when upset</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>65</td>
<td>My child gets full up easily</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>66</td>
<td>My child eats more when s/he has nothing else to do</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
<tr>
<td>67</td>
<td>Even if my child is full up s/he finds room to eat his/her favourite food</td>
</tr>
<tr>
<td></td>
<td>![Rating Scale](Never, Rarely, Sometimes, Often, Always)</td>
</tr>
</tbody>
</table>
68 If given the chance, my child would drink continuously during the day

Never  Rarely  Sometimes  Often  Always

69 My child cannot eat a meal if s/he has had a snack just before

Never  Rarely  Sometimes  Often  Always

70 If given the chance, my child would always be having a drink

Never  Rarely  Sometimes  Often  Always

71 My child is interested in tasting food s/he hasn’t tasted before

Never  Rarely  Sometimes  Often  Always

72 My child decides that s/he doesn’t like a food, even without tasting it

Never  Rarely  Sometimes  Often  Always

73 If given the chance, my child would always have food in his/her mouth

Never  Rarely  Sometimes  Often  Always

74 My child eats more and more slowly during the course of a meal

Never  Rarely  Sometimes  Often  Always

Section 5: Mealtimes

Parents sometimes eat meals together and sometimes do not. In this section, we present statements about mealtimes with your child. Please tick the response that best describes your mealtimes with your child at home. If you disagree or never feel that way, choose “never”. If you agree, let us know how often you feel that way.

75 Mealtimes with my child take a lot of effort

Never  Rarely  Sometimes  Often  Always
76. It distresses me if my child just picks at the food on the plate

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

77. It bothers me when my child refuses to eat a food I prepared

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

78. I become impatient if my child takes too long to finish eating

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

79. I am upset when my child doesn’t eat

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

80. I make sure that my child eats everything on the plate (for example, I feed my child the remaining food)

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

81. I decide when my child has had enough

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

82. I decide what foods my child eats

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

83. I decide when my child eats (for example, a planned time for meals and snacks)

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

84. I decide which snacks my child eats

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Item</td>
<td>Description</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>I make sure that my child tastes all foods served</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>My child decides how much to eat at a meal (my child eats as much as s/he wants)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>My child decides when s/he eats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>My child takes a bite of each food served</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>My child decides if s/he eats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>My child decides when s/he has enough (ie is done eating)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>My child decides what to eat of the foods served (chooses from foods already prepared)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>My child tries new foods (for example, will take a bite or taste of a new food)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>My child refuses vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>I make sure that my child tastes all foods served</td>
</tr>
<tr>
<td>86</td>
<td>My child decides how much to eat at a meal (my child eats as much as s/he wants)</td>
</tr>
<tr>
<td>87</td>
<td>My child decides when s/he eats</td>
</tr>
<tr>
<td>88</td>
<td>My child takes a bite of each food served</td>
</tr>
<tr>
<td>89</td>
<td>My child decides if s/he eats</td>
</tr>
<tr>
<td>90</td>
<td>My child decides when s/he has enough (ie is done eating)</td>
</tr>
<tr>
<td>91</td>
<td>My child decides what to eat of the foods served (chooses from foods already prepared)</td>
</tr>
<tr>
<td>92</td>
<td>My child tries new foods (for example, will take a bite or taste of a new food)</td>
</tr>
<tr>
<td>93</td>
<td>My child refuses vegetables</td>
</tr>
</tbody>
</table>
94. My child is a picky eater (for example, will only eat certain foods)

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

95. My child refuses fruits

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

96. My child accepts new foods

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

97. How often does your child eat his/her main meal alone (child is only one eating)?

<table>
<thead>
<tr>
<th></th>
<th>Never/rarely</th>
<th>Less than once a week</th>
<th>Once a week</th>
<th>Twice a week</th>
<th>Three times a week</th>
<th>Four times a week</th>
<th>Five or more times a week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

98. How often does your child eat a main meal with you or your partner?

<table>
<thead>
<tr>
<th></th>
<th>Never/rarely</th>
<th>Less than once a week</th>
<th>Once a week</th>
<th>Twice a week</th>
<th>Three times a week</th>
<th>Four times a week</th>
<th>Five or more times a week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

99. How often does your family sit down together for a main meal?

<table>
<thead>
<tr>
<th></th>
<th>Never/rarely</th>
<th>Less than once a week</th>
<th>Once a week</th>
<th>Twice a week</th>
<th>Three times a week</th>
<th>Four times a week</th>
<th>Five or more times a week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

100. How often does your child eat approximately the same food as you for the main meal?

<table>
<thead>
<tr>
<th></th>
<th>Never/rarely</th>
<th>Less than once a week</th>
<th>Once a week</th>
<th>Twice a week</th>
<th>Three times a week</th>
<th>Four times a week</th>
<th>Five or more times a week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

101. My child feeds him/herself as expected for his/her age

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Seldom</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
102 I feel confident my child eats enough Is this a problem for you?

Never Seldom Sometimes Often Always Yes No

103 I find our meals stressful Is this a problem for you?

Never Seldom Sometimes Often Always Yes No

104 My child uses cutlery as expected for his/her age Is this a problem for you?

Never Seldom Sometimes Often Always Yes No

105 My child eats chunky foods Is this a problem for you?

Never Seldom Sometimes Often Always Yes No

Section 6: Activities

These questions ask about activities babies may do. Your baby may have already done some of the activities described here, and there may be some your baby has not begun doing yet. If your baby hasn’t tried an activity yet, try it with them now before answering the question.

106 While holding on to furniture does your baby bend down and pick up a toy from the floor and then return to a standing position?

Yes Sometimes Not yet
107 While holding on to furniture, does your baby lower herself with control (without falling or flopping down)?

- Yes
- Sometimes
- Not yet

108 Does your baby walk beside furniture while holding on with only one hand?

- Yes
- Sometimes
- Not yet

109 If you hold both hands just to balance your baby, does he take several steps without tripping or falling? (If your baby already walks alone, mark “yes” for this item).

- Yes
- Sometimes
- Not yet

110 When you hold one hand just to balance your baby, does she take several steps forward? (If your baby already walks alone, mark “yes” for this item).

- Yes
- Sometimes
- Not yet
111 Does your baby stand up in the middle of the floor by himself and take several steps forward?

- Yes
- Sometimes
- Not yet

112 After one or two tries, does your baby pick up a piece of string with his first finger and thumb? *(The string may be attached to a toy).*

- Yes
- Sometimes
- Not yet

113 Does your baby pick up a crumb (or something similar) with the *tips* of her thumb and finger? She may rest her arm or hand on the table while doing it.

- Yes
- Sometimes
- Not yet

114 Does your baby put a small toy down, without dropping it, and then take his hand off the toy?

- Yes
- Sometimes
- Not yet

115 Without resting her arm or hand on the table, does your baby pick up a crumb (or something similar) with the *tips* of her thumb and a finger?

- Yes
- Sometimes
- Not yet
116  Does your baby throw a small toy with a forward arm motion? (*If he simply drops the ball, mark “not yet” for this item*).

- Yes
- Sometimes
- Not yet

117  Does your baby help turn the pages of a book? (*You may lift a page for him to grasp*).

- Yes
- Sometimes
- Not yet

Section 7: General questions

Any finally, just a few questions about yourself and your baby.

118  What is your current employment situation? (*You may choose more than one*)

- I am in full-time paid employment
- I am in part-time paid employment
- I am a full-time student
- I am a part-time student
- I am not in paid employment and am not a student (please go to question 121)
- I am a stay-at-home parent (please go to question 121)

119  What age was your baby when you returned to work or study (for at least 10 hours per week)?

_____ weeks OR _____ months of age
120 Do you think returning to work or study affected how your baby was fed in any way?
- No it didn’t
- Yes it did
If yes, please explain ____________________________________________________________

121 What is your relationship to this baby?
- I am the baby’s mother
- I am the baby’s father (please go to question 123)
- Other, (please state relationship) …………… (please go to question 123)

122 What was your smoking status when you were pregnant with this baby?
- I was a daily smoker
- I was an occasional smoker
- I quit smoking during the pregnancy (please state which month of pregnancy ____)
- I did not smoke when I was pregnant

123 On a scale of 1-10, do you think you are a relaxed or a tense person?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tense</td>
</tr>
<tr>
<td>Middle of the road</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

124 Do you have any comments you would like to make about the study?
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................

125 Do you have any comments you would like to make about the group (‘BLISS’ or ‘Control’) you were in?
........................................................................................................................................
........................................................................................................................................
........................................................................................................................................

279
126 Has your baby been constipated at any time since they started having solids?

○ No   *Thank you – you have finished the questionnaire*

○ Yes   *(please continue)*

127 If yes, how old was baby when it **first** happened (after they started solids)?

______________ months

If it has happened more than once, how old was baby when it most recently happened?

______________ months

128 How would you describe their poo when they were constipated (tick as many boxes as you need to)

○ separate hard lumps like nuts (or pebbles)

○ sausage-shaped but lumpy

○ like a sausage but with cracks on the surface

○ like a sausage or snake but smooth and soft

○ soft blobs

○ mushy

○ watery

○ other (please state) ________________________________

129 Was poo-ing painful when they were constipated?

○ No

○ Yes

130 How often did they do a poo when they were constipated?

○ more than once a day

○ every day

○ 5-6 times a week

○ 3-4 times a week

○ 2 times a week

○ once a week

○ less than once a week

Thank you for completing this questionnaire
Appendix F: BLISS weighed three-day diet record
BLISS - Main food diary

BLISS Food Diary

Please read through the instruction pages before starting your food diary

Things to record each day:

<table>
<thead>
<tr>
<th>What</th>
<th>When</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Food Diary</td>
<td>During preparation, mixing and cleaning up of the food or drink</td>
</tr>
<tr>
<td>2. Description of Recipes Used</td>
<td>As you are cooking the recipe</td>
</tr>
<tr>
<td>3. End of Day Questionnaire</td>
<td>At the end of each day</td>
</tr>
<tr>
<td>4. Supplement</td>
<td>At the end of each day</td>
</tr>
</tbody>
</table>

On these days:

1. ............................................
2. ............................................
3. ............................................

Please try not to change what you give your child just because you are keeping a diary!

Thank you very much for your help

A) How to fill out your food diary

- Answer step 1 to step 6 for everything your child eats and drinks, what eats and drinks it. Please don’t rely on your memory at the end of the day.
- Record the amount and description of ALL foods and drinks consumed — all meals and all snacks.
- Begin each new day on its labelled page (for example, Day 1). Remember each day starts at midnight and ends the following midnight. So please remember to record feeds that occur at night.
- Use a new line for each food or drink. (You can use more than one line for a food or drink, but please start each new food or drink on a separate line).
- Also please remember to include any additions to foods (for example, tomato sauce, salad dressing, gravy).

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of day</td>
<td>Name of food or drink</td>
<td>Brand of food or drink</td>
<td>Cooking method</td>
<td>Weight of food or drink</td>
<td>Weight of food drink item</td>
</tr>
<tr>
<td>Please write down the time your child had something to eat, or drink, including am or pm</td>
<td>Describe the food or drink.</td>
<td>Name the brand.</td>
<td>If the food was cooked, write down how it was cooked (boiled, steamed, fried). If the food was eaten on something or you added things like sauce or butter, please record this. If a recipe was used to make a dish, please write the “see recipe” and write on the page the recipe used.</td>
<td>1) Weigh an empty plate or mug using the scales provided.</td>
<td>1) Place the first food or drink on the plate or mug on the scales.</td>
</tr>
</tbody>
</table>
An example filled out by the parents of a 14 month old toddler

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of day</td>
<td>Name of food or drink</td>
<td>Brand of food or drink</td>
<td>Cooking method</td>
<td>Weight of plate/meal</td>
<td>Weight of food in plate/meal</td>
</tr>
<tr>
<td>7:30am</td>
<td>Breastfeed 15 minutes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>10am</td>
<td>Fruit cake</td>
<td>Buy</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>12pm</td>
<td>Medium size</td>
<td>Buy</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3pm</td>
<td>Breastfeed 20 minutes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>6pm</td>
<td>Potatoes</td>
<td>Boiled</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

B) How to describe recipes

Example:

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of recipe</td>
<td>Amount of each ingredient including any water added (eg. 3 medium carrots, 500g lean beef mince, 1 onion, 60g water etc)</td>
<td>Cooking method</td>
<td>Proportion of recipe served to your child</td>
<td>Time of day served</td>
</tr>
<tr>
<td>Home-made mince</td>
<td>300g standard beef mince (browned in 1 tablespoon olive oil) 50g onion, diced 60g carrot, diced 1 clove garlic, minced 60g beef stock (Campbell’s) 30g tomato sauce (Warities) 60g diced potatoes 40g diced kumara 40g frozen mixed vegetables (Warities) 60g water 5g white flour</td>
<td>Minced was stewed in a small pot with the lid on</td>
<td>One tenth (1/10)</td>
<td>6 pm</td>
</tr>
</tbody>
</table>
C) How to estimate amounts of food when you can’t weigh them

Please record an estimated amount in the “weight of food or drink” column.
- **HOUSING MEASURES** – Household measures like cups, tablespoons and teaspoons can be useful. Please tell us whether it was a heaped or level amount.
- **WEIGHTS MARKED ON PACKAGES** – Use the weight marked on canned or packet foods e.g., half a 220g can of baked beans, one 60g bottle of yoghurt.
- **RULER** – Foods such as cheese, cakes and meat can be measured using the ruler provided in the Away from Home booklet (page 10), e.g. slice of luncheon sausage 8cm x 6cm x 1mm (remember to give length, width and depth!).
- **CIRCLES** – Round foods such as biscuits and muffins can be measured using the circles provided in the Away from Home booklet (page 11), e.g. one muffin 6cm circle x 7cm high (height estimated using the ruler).
- **BREAD** – Tell us the number and the size of the slices e.g., sandwich, medium, or toast slice.
- **FRUIT** – Tell us whether the piece of fruit is small, medium or large. Alternatively you could use the circles for round fruits such as mandarins.

**TAKEAWAY FOODS**
The Away from Home booklet (page 9) has photographs of commonly eaten takeaway foods. Please write down the weight from the photograph that best describes the amount of food your child was served and write it in the “Weight of food or drink” column. Your child might not have exactly the amount in the photos so feel free to tell us if she had “two x 40g pizza.”

Remember: We are NOT looking for a “healthy” diet. We need to know what children actually eat and how they eat it.

---

D) How to fill out your End of Day Questionnaire

**Table 1**: Please answer Step 1 - 4

**Table 2**: Please answer Part 1 or Part 2, depending on you child’s food and drink intake today.

: If you answer Part 2, please fill in all the steps.

### Table 1

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Day of week</td>
<td>Is this a typical eating day for your child?</td>
<td>Is your child unwell for any reason?</td>
</tr>
<tr>
<td>3 March 2019</td>
<td>Thursday</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes - Decreased appetite</td>
<td>Yes - Increased appetite</td>
</tr>
</tbody>
</table>

### Table 2

**An example filled out by the parents of a 9 month old**

**How did your child’s meals compare to the family meals today?**

- If you tick a box in Part 1, you don’t need to in Part 2 (the other box is ticked).  
- If not, fill in both boxes.

**Part 1**

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Lunch</th>
<th>Evening meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Part 2**

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Meal Ingredients</th>
<th>Meal Preparation (size, texture of meal, length and method of cooking, size of food pieces)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- “Similar” could be serving some of the same ingredients, but having new or changing others.
- “Almost the same” could be cooking vegetables a little longer, or eating foods like finger foods instead of covered pieces.
- “Different” could be serving most vegetables and chicken for the family meals, and making similar meals for your child.

---

285
Day 1

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of day</td>
<td>Name of food or drink</td>
<td>Brand of food or drink</td>
<td>Cooking method</td>
<td>Weight of plate or mug</td>
<td>Weight of food or drink</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If your child has finished eating for the day, please remember to fill out the end of day questionnaire and supplement use on pages 11 and 12.

End of Day Questionnaire – Day 1

Table 1

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Day of week</td>
<td>Is this a typical eating day for your child?</td>
<td>Is your child unwell (for any reason)?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2

How did your child’s meals compare to the family meals today?

<table>
<thead>
<tr>
<th>Part 1</th>
<th>Part 2</th>
<th>Part 3</th>
<th>Part 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did not have this meal today</td>
<td>Breast milk or formula and food</td>
<td>Meal Preparation (big texture of meal, length and method of cooking, size of food pieces)</td>
<td>Exact the same, Almost the same, or Similar</td>
</tr>
<tr>
<td>Was with another adult at this meal time</td>
<td>Child ate meal with at least one other adult (both were eating but food may be different)</td>
<td>Meal Ingredients</td>
<td>Yes or No</td>
</tr>
</tbody>
</table>
Supplement Use – Day 1

(a) Did your child take any supplements today? Include anything you consider to be a supplement to your child’s diet (e.g., multivitamin, etc.).

No  □ (please go to page 13)
Yes □

(b) If yes, please record the following:

Type of supplement (e.g., cod liver oil): ______________________
Brand name (e.g., Smith’s): ______________________
Amount (number of mls, drops, tablets, capsules, etc.) taken (e.g., 5ml): ______________________

(c) If yes, does the supplement contain iron or zinc? (check the label)

No  □
Yes □

If yes, please record the type of iron (e.g., ferrous fumarate, ferrous sulphate and anything else with the words “ferrous”, “ferric” or “ferrous” or “zinc” (e.g., zinc sulfate) and the amount of iron or zinc per tablet (e.g., 10mg, etc.):

Type of iron (e.g., ferrous sulphate): ______________________ Amount per dose (e.g., 7mg in 5ml): ______________________

Type of zinc (e.g., zinc sulfate): ______________________ Amount per dose (e.g., 7mg in 5ml): ______________________

THE INTERVIEWER WILL HELP YOU FILL IN THIS PAGE IF YOU ARE NOT SURE – please keep the bottle or packet

B) How to describe recipes

Example:

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of recipe</td>
<td>Amount of each ingredient including any water added (e.g., 2 medium carrots, 500g lean beef mince, 1 onion, 60g water etc)</td>
<td>Cooking method</td>
<td>Proportion of recipe served to your child e.g., one tenth or 1/10 or 10%</td>
<td>Time of day served</td>
</tr>
</tbody>
</table>

12 13
BLISS - Away from home booklet

BLISS Food Diary
Away from Home Booklet

- Please use this booklet if you are leaving the house and will be unable to weigh foods.
- Remember we are NOT looking for a "healthy" diet. We need to know what children actually eat and how they eat.

Thank you very much for your help

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>Step 2</td>
</tr>
<tr>
<td>Time of day</td>
<td>Name of food or drink</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How to estimate amounts of food when you can’t weigh them
Please record an estimated amount in the “weight of food or drink” column.

- HOUSEHOLD MEASURES – Household measures like cups, tablespoons and teaspoons can be useful. Please tell us whether it was a heaped or level amount.
- WEIGHTS MARKED ON PACKAGES – Use the weight marked on canned or packet foods e.g., ½ x 220g can of baked beans, 1 x 60g bottle of yoghurt.
- RULER – Foods such as cheese, cakes and meat can be measured using the ruler provided on page 10, e.g., slice of lunchmeat sausage 8cm x 4cm x 1mm (remember to give length, width and depth).
- CIRCLES – Round foods such as biscuits and muffins can be measured using the circles provided on page 11, e.g., one muffin 6cm circle x 7cm high (height estimated using the ruler).
- BREAD – Tell us the number and the size of the slices e.g., sandwich, medium, or toast slice.
- FRUIT – Tell us whether the piece of fruit is small, medium or large. Alternatively you could use the circles for round fruit such as mandarins.

TAKEAWAY FOODS
Page 9 has photographs of commonly eaten takeaway foods. Please write down the weight from the photograph that best describes the amount of food your child was served and write it in the “weight of food or drink” column. Your child might not have exactly the amount in the photos so feel free to tell us if she had “two x 40g pizza”.

Remember: We are NOT looking for a “healthy” diet. We need to know what children actually eat and how they eat it.
Takeaway Foods Estimation Guide

Fries

Chips

Hawaiian Pizza

Battered Fish

Thank you!

Remember: if you have any questions please contact us. You can email or call our answer phone and we’ll get back to you.

Adapted from Adult Nutrition Survey 08/09

BLISS Study
Department of Human Nutrition University of Otago
P.O. Box 56, Dunedin 9054
Email: bliss@otago.ac.nz
Answer phone: (03) 471 8653

version 2013-2014
BLISS – Childcare food diary

BLISS Food Diary – Childcare

Name of staff member: ___________________________ Date: ___________________________

_________________________ is involved in a study looking at infant feeding practices. We would appreciate it if you could record a complete description of what s/he eats today while in your care, following the instructions below. Your help in completing this food record will contribute to determining the advantages and limitations of a baby-led approach to infant feeding.

We would like you to please:

- **Step 1 & 2:** Record what will be offered to the child today based on the menu or what has been put into their lunch box, and write the time of the day they are served these items. Please list each food or drink item individually (e.g., “bread ‘cheese’ instead of ‘cheese on toast’) and remember to include all water, breast milk and formula as well.

- **Step 3:** Tick the option that best describes the consistency of the food item (e.g., **pureed** to a smooth consistency, **mashed** to a lumpy consistency, **diced** into pieces that need to be eaten with a spoon, or served as a **whole** food).

  NB: Whole may include food that has been cut up into more manageable portion sizes such as sliced toast. Liquids, sauces and spreads are always served in their whole form.

- **Step 4:** Record an **estimate** of how much food and drink s/he has **EATEN**, rather than how much you offered. You can use household measures (e.g., cups or spoons), sizes of packets (e.g., 140g yoghurt pottle, 15g “Little Kids” bar).

- **Step 5:** Tick the option that best describes who put the food in his/her mouth. You can tick both options if it was a combined effort.

- **Step 6:** If any foods eaten are recipes made at the centre, please attach a copy of the recipe to this sheet, including the number of portions the recipe makes. Then in the “amount eaten” column, please record how many of these portions s/he ate e.g., ½ a portion or 2 portions.

Here’s an example of how to fill out the food diary:

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Name</th>
<th>Brand</th>
<th>Cooking method</th>
<th>Consistency of food item</th>
<th>Amount eaten</th>
<th>Food/drink was put in child’s mouth by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 am</td>
<td>Peaches, canned</td>
<td>Oak</td>
<td></td>
<td>Pureed</td>
<td>1/3 cup</td>
<td>Adult: ✓, Child: ✓</td>
</tr>
<tr>
<td></td>
<td>Sugar-free fruit drink</td>
<td>Raro</td>
<td></td>
<td>Mashed</td>
<td>1/4 cup</td>
<td>Adult: ✓, Child: ✓</td>
</tr>
<tr>
<td>12 noon</td>
<td>Lasagne- See Recipe</td>
<td></td>
<td></td>
<td>Diced</td>
<td>1 portion</td>
<td>Adult: ✓, Child: ✓</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>boiled</td>
<td></td>
<td>Whole</td>
<td>1/4 cup</td>
<td>Adult: ✓, Child: ✓</td>
</tr>
<tr>
<td></td>
<td>Frozen peas</td>
<td>Watties</td>
<td>boiled</td>
<td></td>
<td>10 peas</td>
<td>Adult: ✓, Child: ✓</td>
</tr>
<tr>
<td>1:30pm</td>
<td>Fruit bar, Strawberry and</td>
<td>Mother</td>
<td></td>
<td></td>
<td>1 bar (15g)</td>
<td>Adult: ✓, Child: ✓</td>
</tr>
</tbody>
</table>

We know how busy ECE Centres can be - Thank you very much for filling out the information on these pages, we really appreciate your support of the parents’ participation in this study.

If you would like to know more about the Baby-Led Introduction to Solids study (BUSS), our website is: bliss.otago.ac.nz. If you have any comments or questions, then please feel free to contact the study on (03) 471 6063 or bliss@otago.ac.nz.
## Food Diary:

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of day</td>
<td>Name</td>
<td>Brand</td>
<td>Cooking method</td>
<td>Consistency of food item</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Purred</td>
</tr>
</tbody>
</table>

Thank you!
BLISS – Laminated example of a completed food diary day

**An example filled out by the parents of a 14 month old toddler**

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of day</td>
<td>Name of food or drink</td>
<td>Brand of food or drink</td>
<td>Cooking method</td>
<td>Weight of food drink + plate/mug</td>
<td>Consistency of food drink</td>
</tr>
<tr>
<td>7:30am</td>
<td>Breasted 13 minutes</td>
<td>1 slice white bread toast slice</td>
<td>Tip top toasted</td>
<td>115g 135g</td>
<td>✓</td>
</tr>
<tr>
<td>8:30am</td>
<td>Avocado, shelled</td>
<td>Please write down food or drink</td>
<td>Isolated</td>
<td>160g</td>
<td>✓</td>
</tr>
<tr>
<td>8:30am</td>
<td>Banana</td>
<td>Fruit knife</td>
<td>Isolated</td>
<td>162g</td>
<td>✓</td>
</tr>
<tr>
<td>9am</td>
<td>Baby food, peach, apricot and yam sauce</td>
<td>Waffles</td>
<td>microwave</td>
<td>80g 200g</td>
<td>✓</td>
</tr>
<tr>
<td>10am</td>
<td>Fruit cake</td>
<td>If you don't have scales with you please estimate the amount.</td>
<td>2 napkin boxes</td>
<td>127g</td>
<td>✓</td>
</tr>
<tr>
<td>11am</td>
<td>Raw</td>
<td>Small</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>11:30am</td>
<td>Breasted 10 minutes</td>
<td>McDonalds</td>
<td>McDonalds</td>
<td>105g 115g</td>
<td>✓</td>
</tr>
<tr>
<td>12pm</td>
<td>Medium fruit 4lg (from Away from home booklet pg 9)</td>
<td>McDonalds</td>
<td>McDonalds</td>
<td>115g 125g</td>
<td>✓</td>
</tr>
<tr>
<td>2pm</td>
<td>Breaken 20 minutes</td>
<td>McDonalds</td>
<td>McDonalds</td>
<td>105g 115g</td>
<td>✓</td>
</tr>
<tr>
<td>3pm</td>
<td>Avocado and mince (see recipe)</td>
<td>155g</td>
<td>145g</td>
<td>50g</td>
<td>✓</td>
</tr>
<tr>
<td>4pm</td>
<td>Potato</td>
<td>Boiled</td>
<td>195g</td>
<td>197g</td>
<td>✓</td>
</tr>
<tr>
<td>5pm</td>
<td>Peas, frozen</td>
<td>Boiled</td>
<td>211g</td>
<td>211g</td>
<td>✓</td>
</tr>
<tr>
<td>6pm</td>
<td>Fruit juice = Orange and mango</td>
<td>Just juice</td>
<td>42g 210g</td>
<td>42g 210g</td>
<td>✓</td>
</tr>
<tr>
<td>7pm</td>
<td>Ice cream = Vanilla</td>
<td>Tip top</td>
<td>80g 140g</td>
<td>80g 140g</td>
<td>✓</td>
</tr>
<tr>
<td>8pm</td>
<td>Infant formula = follow-on formula</td>
<td>Smiles</td>
<td>50g 250g</td>
<td>50g 250g</td>
<td>✓</td>
</tr>
</tbody>
</table>

**D) How to fill out your end of Day Questionnaire**

Table 1: Please answer all steps (Step 1 - 4).

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Is this a typical eating day for your child?</td>
<td>Is your child unwell for any reasons?</td>
<td>If unwell, did this influence your child’s appetite?</td>
</tr>
<tr>
<td>2 March 2013</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 2: Please answer Part 1 or Part 2, depending on you child’s food and drink intake today.

<table>
<thead>
<tr>
<th>Part 1</th>
<th>Part 2</th>
<th>Part 3</th>
<th>Part 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didn't have this meal today</td>
<td>Breakfast milk formula and food</td>
<td>Child ate meal with at least one other adult</td>
<td>Meal ingredients</td>
</tr>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Similar to occurring some of the same ingredients, but language or name changing others.</td>
</tr>
<tr>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Similar to occurring some of the same ingredients, but also different to each other.</td>
</tr>
</tbody>
</table>

292
Appendix G: Weighed diet record calculation sheet and entry protocol
<table>
<thead>
<tr>
<th>Food item</th>
<th>Weight of plate</th>
<th>Weight of food + plate</th>
<th>Weight of leftover + plate</th>
<th>Total weight of food offered</th>
<th>Weight of individual food items offered</th>
<th>Total weight of leftover foods</th>
<th>Total weight of foods eaten</th>
<th>Factor to multiply food items by</th>
<th>Weight of food item CONSUMED</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D = (B total) - A</td>
<td>E1 = B - A</td>
<td>E2 = B - (B of food above)</td>
<td>F = C - A</td>
<td>G = D - F</td>
<td>H = G/D</td>
<td>I = H*E</td>
</tr>
</tbody>
</table>

1. Example of combined foods with a plate and NO leftovers

| bread     | 100g           | 142g                  | 142-100 = 42g            |                             |                                      | 1*42= 42g                      |                            |                             |                            |
| spread    | 152g           |                       | 152-142 = 10g            |                             |                                      | 1*10 = 10g                     |                            |                             |                            |
| jam       | 157g           |                       | 157-152 = 5g             |                             |                                      | 1*5 = 5g                       |                            |                             |                            |
| banana    | 283g           | 283-100 = 183g       | 283-157 = 126g           | 0                           | 283-100 = 183                     | 183/183 = 1.00                | 1*126 = 126g               |                            |

2. Example of combined foods with a plate and leftovers

| bread     | 100g           | 142g                  | 142-100 = 42g            |                             |                                      | 0.73*42 = 30.66g              |                            |                             |                            |
| spread    | 152g           |                       | 152-142 = 10g            |                             |                                      | 0.73*10 = 7.30g               |                            |                             |                            |
| jam       | 157g           |                       | 157-152 = 5g             |                             |                                      | 0.73*5 = 3.65g                |                            |                             |                            |
| banana    | 283g           | 283-100 = 183g       | 283-157 = 126g           | 150-100 = 50g              | 183-50 = 133                     | 133/133 = 0.73                | 0.73*126 = 91.96g          |                            |

3. Example of combined foods with NO plate and leftovers

| bread     | 42             |                       | 42                        |                             |                                      | 0.73*42 = 30.66g              |                            |                             |                            |
| spread    | 52             |                       | 52-42 = 10g               |                             |                                      | 0.73*10 = 7.30g               |                            |                             |                            |
| jam       | 57             |                       | 57-52 = 5g                |                             |                                      | 0.73*5 = 3.65g                |                            |                             |                            |
| banana    | 183            | 183g                  | 183-57 = 126g             | 50g                         | 183-50 = 133                     | 133/133 = 0.73                | 0.73*126 = 91.96g          |                            |
WDR entry protocol – used for WDRs collected at both 7 and 12 months

### Protocol for 7-month BLISS diet record Kai-calculator data entry

**Copy no. _____**

<table>
<thead>
<tr>
<th>Study:</th>
<th>BLISS Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepared by:</td>
<td>Liz Williams</td>
</tr>
<tr>
<td></td>
<td><strong>Version number:</strong> Version 3</td>
</tr>
<tr>
<td></td>
<td><strong>Date prepared:</strong> 17 Aug 14</td>
</tr>
</tbody>
</table>

### Purpose of diet record entry data protocol

**Objective:** To ensure accurate and consistent entry of BLISS diet record data.

### Equipment required

**Protocols:** Protocol for 7-month BLISS diet record Kai-calculator data entry.

(This protocol)

**Documents:**

- “BLISS CODEbook” excel file (access via BLISS google docs)
- “New foods and substitutions request” excel file (access via BLISS google docs)
- “BLISS diet record eating occasions definitions” (download from BLISS dropbox)
- “BLISS diet record calculations examples” (download from BLISS dropbox)
- “BLISS_7month-3DDR data entry tracking” (download from BLISS dropbox)
- BLISS 3 day diet record
- Away from home booklet (if used by participant)
- Early childhood education food diary (if used by participant)
- Diet record instructions (written and tested by Claire Schramm)

**Equipment:**

- Coloured pens
- Calculator
  - “BLISS diet record calculations” (download from BLISS dropbox and print to staple into diet record)
  - “BLISS diet record RECIPE calculations” (download from BLISS dropbox and print to staple into diet record if required)

**ALL notes/marks added to the original diet record MUST be in a different pen colour (e.g. PINK) and the name of the person who made**
the notes and the date they were made MUST be recorded on the front of the diet record with the colour of the pen (or a scribbled sample of the colour)

Steps – First pass

1. **Calculate the weight of each food item OFFERED and CONSUMED by the infant using the “BLISS diet record calculations”:**

   - **Step 3** of the BLISS diet record (Weight of plate) AND (Weight of food + plate)
   - **Step 6** of the BLISS diet record (Weight of leftover + plate) OR/AND (Estimation of what is left on plate)

   **NOTE:** ‘COMBINED foods’ are food items that were weighed progressively as a group (mark each group with brackets on the left hand side of the calculations page and on the original diet record)

   ‘INDIVIDUAL foods’ are foods that were weighed on their own

1. Enter all values provided on the diet record onto the calculations page.
   - If a plate/cup/bottle was used enter “Weight of plate”, “Weight of food + plate” and “Weight of leftover + plate” into “BLISS diet record calculations” columns A, B and C from the diet record.

   - If no plate/cup etc was used the weight from **STEP 3** (Weight of food + plate) can be entered directly into column D of the calculations page and the “Weight of leftover + plate” into column F (when no “Weight of plate” is provided the “Weight of leftover + plate” is assumed to equal the “weight of leftover foods”).

   - If there were no leftovers (assumed if no “Weight of leftover + plate” or “Estimation of what is left on plate” is provided) enter 0 into column F.

   - When there is no weight for a food in the diet record, the weight has to be estimated.
• When estimated quantities have been provided (i.e. household measures like a 1 cup or 1 TBsp or size measures like a medium apple or one piece of toast) the “Food weight estimation DEFAULTS” sheet from the “BLISS CODEbook” contains weights extracted from Kai-culator to be used to estimate the weight of food offered.

• Foods that have no measures to guide estimation are (where possible) estimated using quantities of the food reported to be consumed by the infant on other occasions OR (as a last resort) by other infants of the same age in other diet records.

• When a weight estimation for a food is not available in the “Food weight estimation DEFAULTS” sheet it should be added to the “New foods and substitutions request” document. Once an estimation quantity has been chosen (i.e. 2 grams of margarine on 1 piece of toast) it is entered into the “Food weight estimation DEFAULTS” sheet in the “BLISS CODEbook” google doc to ensure consistent use across all BLISS diet record diet record projects.

• When a single overall weight has been given for COMBINED foods (i.e. toast with margarine and marmite) the weights for the individual food items are estimated using weights from the “Food weight estimation DEFAULTS” sheet.

2. Calculate the “Total weight of food offered” (not needed when no plate was used as this will be entered directly from the diet record):

For INDIVIDUAL foods:

\[ B \text{ (Total weight of food + plate)} - A \text{ (Weight of plate)} = D \text{ (Total weight of food offered)} \]

For COMBINED foods:

\[ B \text{ (Total weight of food + plate)} \text{ OF THE LAST FOOD ADDED} - A \text{ (Weight of plate)} = D \text{ (Total weight of ALL foods offered)} \]

• When baby food was eaten directly from a pouch, jar or can/tin the weight of the pouch, jar or can/tin must be estimated in order to calculate the weight of the baby food OFFERED. Baby food pouch, jar and can weights can be found in the “Food weight estimation DEFAULTS” sheet from the “BLISS CODEbook”. 
3. Calculate the “Weight of individual food items offered” (for COMBINED foods only):

If there is a “Weight of plate” supplied:
B1 (Weight of (first) food + plate) – A (Weight of plate)
= $E_1$ (weight of (first) individual food item offered)

B2 (Weight of (second) food + plate) – B1 (Weight of (first) food + plate)
= $E_2$ (weight of (second) individual food item offered)

etc........

If there is NO “Weight of plate” supplied (i.e. the weights provided were for food only):
B1 (Weight of (first) food + plate)
= $E_1$ (Total weight of (first) food offered)

B2 (Weight of (second) food + plate) - B1 (Weight of food + plate)
= $E_2$ (Total weight of (second) food offered)

etc.....

4. Calculate the “Total weight of leftover foods” (when no plate was used this is entered directly from the diet record):

C (Weight of leftover + plate) - A (Weight of plate)
= $F$ (Total weight of leftover foods)

If there is no (Weight of leftover + plate) supplied:
- Use (Estimation of what is left on plate) in order to calculate the (Weight of leftover food)
  • If a proportion was supplied (i.e. ¼) use it to calculate the proportion of the food item CONSUMED:
    1 – (Proportion of food leftover) = (Proportion of the food item CONSUMED)
    (Proportion of the food item CONSUMED) * (Weight of food OFFERED)
    = (Weight of food item CONSUMED)
If there is no (Weight of leftover + plate) OR (Estimation of what is left on plate) supplied:
It is assumed that (Weight of food item OFFERED) = (Weight of food item CONSUMED)

When a rough approximation has been given for the leftovers it can be converted to an estimation of the amount of the food item left over as below

“a little bit” OR “a tiny bit” = 25%
“Some” = 50%
“Most” = 75%
“Almost all” = 90%

5. Calculate the “Total weight of foods eaten”

If there is a “Weight of leftover + plate” supplied:

\[
D \text{ (Total weight of food offered)} - F \text{ (Total weight of leftover foods)} = G \text{ (Total weight of foods eaten)}
\]

If there is NO “weight of leftover + plate” supplied:

\[
D \text{ (Total weight of food offered)} = G \text{ (Total weight of foods eaten)}
\]

6. Calculate the “Factor to multiply individual food items” (for COMBINED foods only):

\[
G \text{ (Total weight of foods eaten)} \div D \text{ (Total weight of food offered)} = H \text{ (Factor to multiply individual food items)}
\]

When different proportions of combined food components are left over (e.g. chicken ½, potato 1/3, carrot ¼) use the estimated left over proportions to guide calculation of leftover weights (BUT often the estimated leftover proportions overestimate how much of the food components have been consumed in comparison to the total leftover weight)

7. Calculate the “Weight of food item CONSUMED”:

For INDIVIDUAL foods:
\[ G \text{ (Total weight of foods eaten)} = I \text{ (Weight of food item CONSUMED)} \]

For COMBINED foods:
\[ H \text{ (Factor to multiply individual food items)} \times E1 \text{ (weight of (first) individual food item offered)} \]
\[ = I \text{ (Weight of (first) food item CONSUMED)} \]

\[ H \text{ (Factor to multiply individual food items)} \times E2 \text{ (weight of (second) individual food item offered)} \]
\[ = I \text{ (Weight of (second) food item CONSUMED)} \]

etc...

8. Repeat for all foods from DAY 1 of the diet record.
- Mark the end of the day with a line after the last food
- Under the ‘end of day line’ report the:
  • Total number of breastfeeds for infants fed breast milk (remember that feeds with 30 minutes or less between them count as a single feed)
  • Total weight of formula consumed for infants fed infant formula
  • Calculate the weight of breast milk consumed per feed using
    \[ ((750g – \text{weight of formula consumed if any})/\text{number of breastfeeds}) \]

If more than one calculations page is used per diet record day note the page number and total pages at the top of each page (e.g. page 1 of 2, page 2 of 2)

**Staple calculation pages into the original diet record**
2. Recipes
(Mark the food items which are recipes on the diet record calculations sheet for each day for ease of entry)

IF:
All recipe ingredients were weighed and uncooked weights were provided for all ingredients AND a ‘proportion of recipe offered to you child’ was recorded AND the weight of the recipe offered to the child was recorded.
1. If weights have been provided for all recipe ingredients then nothing further is required in preparation for entry into Kai-culator.

IF:
Some/all recipe ingredients were NOT weighed and estimated amounts were provided for all/some ingredients AND a ‘proportion of recipe offered to you child’ was recorded AND the weight of the recipe offered to the child was recorded.
1. When weights have not been provided for some or all ingredients in a recipe, the weight for those ingredients has to be estimated using estimation rules in the “BLISS CODEbook” from the ‘Food weight estimation RULES’ sheet and the ‘Food weight estimation DEFAULT’ sheet.
2. If needed a recipe calculations page can be used to record the estimated weight of each ingredient.
3. The weight of the recipe offered (from the diet record day calculation page) and the ‘proportion of recipe offered to you child’ (from the recipe page e.g. 1/5th) can be used to calculate the approximate weight of the complete cooked recipe

\[
\text{Weight of the recipe offered } \times \text{ proportion of recipe offered to child} = \text{ Approx total cooked weight}
\]

IF:
Some/all recipe ingredients were NOT weighed and estimated amounts were provided for all/some ingredients AND a ‘proportion of recipe offered to you child’ was NOT recorded AND the weight of the recipe offered to the child was recorded.
1. When weights have not been provided for some or all ingredients in a recipe, the weight for those ingredients has to be estimated using estimation rules in the “BLISS CODEbook” from the ‘Food weight estimation RULES’ sheet and the ‘Food weight estimation DEFAULT’ sheet.

302
estimation RULES’ sheet and the ‘Food weight estimation DEFAULT’ sheet.

2. If needed a recipe calculations page can be used to record the estimated weight of each ingredient.

IF:
Some/all recipe ingredients were NOT weighed and estimated amount were provided for all/some ingredients AND a “proportion of recipe offered to your child” was recorded AND the weight of the recipe offered to the child was NOT recorded.

1. When weights have not been provided for some or all ingredients in a recipe, the weight for those ingredients has to be estimated using estimation rules in the “BLISS CODEbook” from the ‘Food weight estimation RULES’ sheet and the ‘Food weight estimation DEFAULT’ sheet.

2. A recipe calculations page should be used to record the estimated weight of each ingredient.

3. Use the “proportion of recipe offered to your child” and the calculated total cooked weight of the receipt from Kai-culator to estimate the weight of the recipe offered to the baby in the diet record day.

Repeat for days 2 and 3 of the diet record

Update the “BLISS 7month-3DDR data entry tracking” document (in dropbox) by recording that the calculations have been done with the name and date of research team member who completed them.
**Steps – Second pass**

**Enter participant RECIPES from Days 1, 2 and 3 into Kai-culator**

1. Open Kai-culator dietary assessment software
2. Open “BLISS study 2014 7 months” project
3. Under “Composition Data” on the side bar select “Recipes”
4. Add a new recipe using the + button at the bottom of the screen
5. Name the recipe using the participant ID followed by the name of the recipe (e.g. BL0623CM - homemade fruit)
6. Enter ingredients and amounts from the recipes page and recipe calculation page
7. Choose an appropriate “moisture retention” value for the whole recipe using moisture retention factors used in similar Adult Nutrition Survey recipes in the Kai-culator database. If there are no similar recipes in the Kai-culator database use the USDA Moisture Retention Factors that can be found in the Kai-culator “Main menu” under “Composition data” in “Moisture factors”.
8. Enter the cooking method, time cooked for and temperature at the top of the recipe ingredients list.
9. Assign a retention definition to each individual ingredient in the recipe by clicking on the rightmost section of the ingredients entry in the recipe screen and choosing the most appropriate option from the menu. Fat’s and sugar do not require retention factors and so can be left as “not applicable ()”.
10. Once the recipe is complete use the calculate button to calculate the nutrient values per 100g for the final recipe.
11. Save and exit the “recipe database”
12. Go to the “food items” list and search for the newly entered recipe, click to select the recipe and use the “paste new item” button and “supress old recipe” option to convert the HN recipe to a ZZ recipe ready to be used when entering a diet record.
**Enter participant DIET RECORD DAYS 1, 2 and 3**

**NOTE:**

A “**substitution**” for a food item is when there is NO food item in Kai-culator that is closely related to the diary food item (e.g. chicken bacon would have to be substituted with pork bacon etc)

A “**default**” for a food item is when there is a food item in Kai-culator that is closely related to the diary food item (e.g. Pams white bread and Bread, white, sliced, prepacked or )

1. Open Kai-culator dietary assessment software
2. Open “BLISS study 2014 7 months” project
3. Open Records
4. Add a new record
5. “Record ID” = BLISS study participant ID -  **(Make sure to double check this step as this cannot be changed!!)**
   “Day No” = 1, 2, or 3
   “Record date” = date that Day 1 was recorded on
6. Using the definitions in the “BLISS diet record eating occasions definitions” document, mark the breakfast meal (BF), lunch (L) and evening meal (EM) onto the diet record.
7. Enter all food items into the “Diary item” quick list, in the order that they were consumed, with the appropriate feeding occasion (refer to “**BLISS diet record eating occasions definitions**” document to determine which eating occasion should be used for each food item – NOTE: the eating occasion can be changed later if required)
8. Match each food item to the best food code from the Kai-culator database

**If complete food item information has been supplied (Brand, flavour etc)**
- Match by brand and flavour/type (i.e light, reduced fat etc) to a food code in Kai-culator
- If there is no exact match refer to “**BLISS CODEbook**” document to check for established DEFAULT
- rules for the food item
• If there is a match in the “BLISS CODEbook” document use the DEFAULT food code.
• Update the “BLISS CODEbook” document substitution entry with:
  • Count of times the DEFAULT has been used
  • BLISS study participant ID for future reference
  • If there is no established DEFAULT rule, enter the food item with all possible details into the “New foods and substitutions request” document

**If the food item information supplied is incomplete**
• Establish WHICH food item data is missing (i.e. Brand missing but flavour/type supplied (e.g. edam cheese) OR Brand and flavour supplied but no type – fresh and fruity berry yoghurt (?? Full fat/light/etc)
• Refer to “BLISS CODEbook” document to check for established DEFAULT rules for the food item
• If there is no DEFAULT rule for the food item enter the food item with all details into the “New foods and substitutions request” document

**If there is no food NO food item in Kai-culator that is closely related to the diary food item (e.g. chicken bacon would have to be substituted with pork bacon etc) a SUBSTITUTION will have to be established**
• When a SUBSTITUTION is used it has to be marked in Kai-culator by ticking in the 7th column on the diet record day.

Check “New foods and substitutions request” for infant foods and infant formulas that are not present in Kai-culator.
• For foods that have already been requested, update the request count and add the BLISS participant ID.
• For foods that have not been requested - add a new food request line to the “New foods and substitutions request” document

**Recipes**
- Use the participant ID to search for previously entered recipes

**COMBINED foods**
- Foods that are eaten as one item (e.g. toast with margarine and jam or cereal with milk) should be linked together in the “food diary reconciliation” page.
- Click on the food diary number in column 1 of the second food of the group of linked foods (e.g. margarine) and select the first food (toast) from the drop down list.

Enter the (Weight/Amount of food item consumed) in the amount column using appropriate units (grams or mL)

Click on the final column of the “food diary reconciliation” page to enter the BLISS supplementary options

- **Who fed the child:**
  Breastfeeds require co-operation between the mother and infant: child + adult
- **Texture of the food:**
  “Naturally smooth” (foods that have to have to have a smooth texture to be themselves):
  - yoghurt, humus, custard, butter, margarine, marmite, vegemite, jam, peanut butter, cream cheese etc
  “Liquid”
  - breast milk, infant formula, milk, water, oil etc

- **Amount offered in grams:** (from column D or E of the diet record calculation page)

  When amounts in the diet record were in mL (e.g. formula or expressed BM) they can be converted to grams by multiplying the amount in mL by the density (g/mL). (Found in the “Measure definitions” under food items)

- **Save and exit record (begin new record for each day of the food diary)**

<table>
<thead>
<tr>
<th>Steps - After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Update the “<strong>Participant diet record data entry tracking</strong>” file</td>
</tr>
</tbody>
</table>
Appendix H: Protocol for checking weighed diet records
Protocol for weighed diet record checking

Study: BLISS Study
Prepared by: Lisa Daniels, Sabina Bacchus
Version number: Version 2
Date prepared: 1 Dec 14
Date amended: 29 Jul 15

Equipment required

- Access to Kai-calculator.
- Access to BLISS dropbox ‘Weighed Diet Records’ folder.
  a. 7 month WDR checking folder or 12 month WDR checking folder
  b. Diet record data entry documents
     i. BLISS ‘CODEbook’ (use the latest version)
     ii. Substitutions and New Foods requests (use the latest version)
  c. WDR checking protocol, templates, and codes folder
     i. WDR checking template
     ii. WDR ingredient checking template
     iii. WDR codes for checking
     iv. Checking sheet _Inside food diaries
- Use a red coloured pen for all checking.
- Hardcopy of weighed diet record and any additional booklets (away from home or childcare).

Calculation checking

1. Check that all foods from each day have been included in each day’s calculation sheet.

2. Check that the correct data from the weighed diet record have been entered in the calculation sheet.

3. Check each calculation for any inaccuracies.

4. Corrections should be made on the calculation sheet in red pen.
Extracting data

1. Open Kai-culator and select the correct project.

2. Select the weighed diet record that you wish to check.

3. Export the diet record by clicking the 'export records' button. The record will be exported as a CSV file.

4. Save it to Dropbox (either in the folder ‘7 month WDR checking’ or ‘12 month WDR checking’) as an Excel Workbook with the BLISS ID as the file name.

5. Using the 'WDR checking template' (Appendix A) found in the 'Weighed Diet Records' folder on Dropbox, copy and paste the relevant column titles from the weighed diet record file.

6. Copy the column titles to the beginning of each food diary days recorded (e.g. day 1, day 2 and day 3).

7. Save this file to Dropbox with the BLISS ID (either in the folder ‘7 month WDR checking’ or ‘12 month WDR checking’) and add 'check' as the file name eg. 'BL1212ZZ check'.

8. Print this file by day.

Recipes

If the weighed diet record includes recipes within the diary these will download as a separate 'ingredients' file when you export the record:

1. Save the ingredients file to Dropbox as an Excel Workbook with the weighed diet record ID and 'ingredients' as the file name eg. 'BL1212ZZ ingredients'.

2. Using the 'WDR Ingredient checking template' (Appendix B) copy and paste the relevant columns from the ingredient file.

3. Save it with the BLISS ID and add 'ingredient check' as the file name eg. 'BL1212ZZ ingredient check'.
4. Print this file.

If the weighed diet record does not include recipes within the diary:

1. Individually check each recipe entered.

2. Go to Recipes, find the name of the recipe, open recipe.

3. Check each ingredient, food weight, retention, cooking method, time and temperature have been correctly entered.

---

### Weighed diet record checking

1. For each food in the weighed diet record, check that it matches the data in your checking file printout and ingredient checking printout, if applicable.

   a. To check the ‘time’, ‘who fed the child’ and ‘consistency’ data see the ‘Codes for checking’ document.

   b. To check default foods and food weight estimations see the ‘BLISS CODEbook’ document.

2. Any revisions to the data should be made in red pen in the applicable ‘revised’ columns.

---

### Making changes in Kai-culator

1. Open Kai-culator and select the correct project.

2. Double click on the weighed diet record you wish to make changes to.

3. To change the ‘descriptor’, click on the item in the ‘diary item’ column. Click SAVE.

4. To change the ‘time’, ‘food item’, or ‘amount’, click on the item in the relevant column. Click SAVE.
5. To change who fed the child (extra 1), consistency of the food (extra 2) or amount offered (extra 4) click on the green tick in the ‘More’ column. Click SAVE.

6. When all changes have been made, save and exit the weighed diet record.

**Final steps**

1. Once the weighed diet record is checked, upload the documents to the relevant WDR checking folder (7 month or 12 month) in the ‘Weighed Diet Records’ folder on Dropbox.

   **Example documents to upload**
   
   BL1212ZZ
   BL1212ZZ check
   BL1212ZZ ingredients
   BL1212ZZ ingredients check

2. Staple the hardcopies of the checking files to the inside of the diet record.

3. Print out the 'Checking sheet_Inside food diaries' document found in the 'Weighed Diet Records' folder in Dropbox, fill out and staple to the inside of the front of the diet record on top of the checking files.

4. On the front of the weighed diet record write your initials in your individual coloured pen.
### Appendix A

Checking document – Example columns

<table>
<thead>
<tr>
<th>id</th>
<th>day</th>
<th>descriptor</th>
<th>time</th>
<th>revised time</th>
<th>fooditem</th>
<th>revised food</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL1212ZZ</td>
<td>1</td>
<td>EGG</td>
<td>AB011</td>
<td></td>
<td>Egg, scrambled, plain</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>amount</th>
<th>revised amount</th>
<th>extra 1 (who fed)</th>
<th>extra 1 revised</th>
<th>extra 2 (consistency)</th>
<th>extra 2 revised</th>
<th>extra 4 (amt offered)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.67</td>
<td>E1002</td>
<td></td>
<td></td>
<td>E2003</td>
<td></td>
<td>15.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>extra 4 revised</th>
<th>date modified</th>
<th>who</th>
<th></th>
</tr>
</thead>
</table>
### Appendix B

Ingredient checking document – Example columns

<table>
<thead>
<tr>
<th>id</th>
<th>recipe</th>
<th>ingredients</th>
<th>food item</th>
<th>revised food item</th>
<th>amount</th>
<th>revised amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL1212ZZ</td>
<td>1</td>
<td>POTATO</td>
<td>Potato, assorted variety, flesh, boiled</td>
<td></td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
Appendix I: Estimation of breast milk intakes at 12 months of age
Estimating breast milk intakes at 12 months of age

It is particularly difficult to estimate breast milk intakes at 12 months of age, as intakes appear to vary from toddler to toddler due to differences in the intakes of complementary foods and other fluids at this age (Kent et al., 1999). Studies reported in Table Appendix I were used to determine the most appropriate estimation of breast milk intakes for participants in the current study (12 months of age). Few studies in high-income countries have determined breast milk intakes in this age group (Dewey et al., 1984; Nommsen et al., 1991; Dewey et al., 1991a), all of which are limited by their small sample sizes. These studies appear to agree that breast milk intakes are between 448 and 567 grams per day at 12 months, as estimated by using test-weighing methods (Table Appendix I). No studies were found in high-income countries assessing estimated breast milk intakes of infants at 12 months of age using the deuterium oxide stable-isotopes method. Other studies have used this available literature to estimate breast milk intakes of infants in their respective studies (Skinner et al., 1997; Devaney et al., 2004; Ponza et al., 2004; Wall et al., 2008; Briefel et al., 2010; Sharma et al., 2013).

Infant formula intake is easily measured compared with breast milk intake, and may be able to give us an indication of the total amount of infant milk consumed by very young children. Formula intakes of BLISS study infants (7 months of age) who were having infant formula but no breast milk were 738 g/day (Williams-Erickson, 2015), which is very similar to the estimated breast milk volume of 750 g/day reported by Dewey et al. (1991a), at 7 months of age. Formula intakes of toddlers in the BLISS study at 12 months of age who were having infant formula but no breast milk were between 330-415 g/day (Chapter 4, Table 4.10), which is also very similar to the estimated breast milk intakes of 448 g/day by Dewey et al. (1991a) at 12 months of age. Therefore, 448 g/day was considered by the Candidate to be the most appropriate estimate of total infant milk intake (breast milk and/or formula) at 12 months of age.
### Table Appendix I. Studies in high-income countries which measured breast milk intakes at 12 months of age

<table>
<thead>
<tr>
<th>Reference and country</th>
<th>Participants</th>
<th>Measurement type and period of measurement</th>
<th>Estimated BM intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dewey et al. (1984)</td>
<td>n=11</td>
<td>Test weighing: over 24hrs. Converted intake to grams and multiplied by 0.983 mL/g to adjust for the density of the BM.</td>
<td>Classified BM volume into 3 tiers (7-16 months): Full lactation: &gt;500 mL/day, n=11, average age = 9.2 months. Partial weaning: 300-500 mL/day, n=6, average age = 10.9 months. Weaning: &lt;300 mL/day, n=6, average age = 13.2 months. Final average BM intake: 550 mL/d = ~567 g/day (11-16 months)</td>
</tr>
<tr>
<td>United States</td>
<td>Breastfeeding women - who breast fed for longer than 6 months.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nommsen et al. (1991)</td>
<td>n=21</td>
<td>Test weighing: over 24hrs for 4 days (at 12 months). Breast milk intake corrected for IWL for each infant to correct for potential error.</td>
<td>Final average BM intake: 514 g/day (12 months)</td>
</tr>
<tr>
<td>United States</td>
<td>Breastfeeding women – who breastfed up to 12 months of age. Criteria: 1) no chronic illness or medication in mother and infant, 2) no solids before 4 months, 3) did not plan to feed their infant &gt;120 mL other milk or formula in first 12 months.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dewey et al. (1991a)</td>
<td>n=42</td>
<td>Test weighing: over 24hrs for 4 days (at 12 months). Breast milk intake corrected for IWL for each infant to correct for potential error.</td>
<td>Final average BM intake: 448 g/day (12 months)</td>
</tr>
<tr>
<td>United States</td>
<td>Breastfeeding women - who breastfed up to 12 months of age. Criteria: 1) Women did not plan to feed their infant &gt;120 mL other milk or formula in first 12 months, 2) no solids before 4 months, 3) infant was healthy, and 4) no chronic illness or medication in mother.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BM, breast milk; IWL, insensible water losses
Appendix J: Contribution of individual foods to food groups
Table Appendix J. Examples of how some of the 1,682 individual foods (rows) were assigned ‘nutrient’ values for Energy (kJ), Iron (fe), Selenium (se), and Zinc (zn) (columns) for the analysis of the ‘nutrient’ contribution of foods to each food group (16 food groups)

<table>
<thead>
<tr>
<th>Descritor</th>
<th>Food id</th>
<th>Breast_g</th>
<th>Breast_kj</th>
<th>Breast_fe</th>
<th>Breast_se</th>
<th>Breast_zn</th>
<th>Infntfor_g</th>
<th>Infntfor_kj</th>
<th>Infntfor_fe</th>
<th>Infntfor_se</th>
<th>Cows_g</th>
<th>Cows_kj</th>
<th>Cows_se</th>
<th>Cows_zn</th>
<th>Bread_g</th>
<th>Bread_kj</th>
<th>Bread_fe</th>
<th>Bread_se</th>
<th>Bread_zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, human, mature</td>
<td>F53</td>
<td>100</td>
<td>288</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>268</td>
<td>0.94</td>
<td>1.48</td>
<td>0.56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infant formula, Heinz Nurture Gold follow on (2), prepared</td>
<td>2277134</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>268</td>
<td>0.94</td>
<td>1.48</td>
<td>0.56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infant formula, Heinz Nurture Gold toddler (3), prepared</td>
<td>22308111</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>287</td>
<td>1.27</td>
<td>0.0001</td>
<td>0.49</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infant formula, Karicare Aptamil Gold Pepti-junior (all ages), prepared</td>
<td>22801060</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>267</td>
<td>0.75</td>
<td>1.36</td>
<td>0.49</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Milk, Anchor, light blue</td>
<td>43894</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Milk, evaporated, whole</td>
<td>F32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Milk, fluid, whole</td>
<td>F33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Milk, farmgate, standard</td>
<td>R4049</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bread, multi-grain light</td>
<td>R4659</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>989</td>
<td>1.37</td>
<td>8.24</td>
</tr>
<tr>
<td>Bread, white, sliced, prepacked</td>
<td>A1007</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oats, rolled, toasted, new zealand</td>
<td>E1015</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cracker, assorted, flavours</td>
<td>A115</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flour, wheat, whole meal</td>
<td>E46</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pasta, vegetable flavour, boiled</td>
<td>E119</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: Bread, ‘breads and cereals’ food group; Breast, ‘breast milk’ food group; Cows, ‘cow’s milk’ food group; Infntfor, ‘Infant formula’ food group
Table Appendix J. (continued) Examples of how some of the 1,682 individual foods (rows) were assigned ‘nutrient’ values for Energy (kJ), Iron (Fe), Selenium (Se), and Zinc (Zn) (columns) for the analysis of the ‘nutrient’ contribution of foods to each food group (16 food groups)

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Food id</th>
<th>Inftcr_e</th>
<th>Inftcr_kj</th>
<th>Inftcr_fe</th>
<th>Inftcr_se</th>
<th>Fruit_g</th>
<th>Fruit_kj</th>
<th>Fruit_se</th>
<th>Fruit_zn</th>
<th>Vege_g</th>
<th>Vege_kj</th>
<th>Vege_fe</th>
<th>Vege_se</th>
<th>Vege_zn</th>
<th>Redme_g</th>
<th>Redme_kj</th>
<th>Redme_fe</th>
<th>Redme_se</th>
<th>Redme_zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farex Baby rice cereal - powder</td>
<td>ZZ609383</td>
<td>...</td>
<td>100</td>
<td>1614</td>
<td>21.6</td>
<td>3.42</td>
<td>3.12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Farex Muesli with apple</td>
<td>ZZ347084</td>
<td>...</td>
<td>100</td>
<td>1709</td>
<td>27.16</td>
<td>4.84</td>
<td>2.12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Farex Original multigrain cereal - powder</td>
<td>ZZ297957</td>
<td>...</td>
<td>100</td>
<td>1605</td>
<td>22.5</td>
<td>3.45</td>
<td>2.09</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Apple, combined cultivars &amp; fresh</td>
<td>L38</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Banana, fresh &amp; fresh</td>
<td>L32</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Currant, dried</td>
<td>L49</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fruit salad, fruit and syrup, canned</td>
<td>L74</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Juice, apple &amp; bilcur, golden circle</td>
<td>C137</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>181</td>
<td>0.07</td>
<td>0</td>
<td>0.03</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Asparagus, canned, drained</td>
<td>X134</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>104</td>
<td>1.42</td>
<td>2.8</td>
<td>0.61</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carrot, fresh, boiled, drained</td>
<td>X33</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>114</td>
<td>0.3</td>
<td>0.12</td>
<td>0.27</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cucumber, fresh, raw</td>
<td>X45</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>43</td>
<td>0.3</td>
<td>0.09</td>
<td>0.16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kumara, baked/ roasted, with no fat</td>
<td>R2475</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>516</td>
<td>0.61</td>
<td>0.14</td>
<td>0.24</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Potato, boiled/ steamed</td>
<td>R1993</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>331</td>
<td>0.31</td>
<td>0.44</td>
<td>0.22</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beef, composite cuts, lean, cooked</td>
<td>M544</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>751</td>
<td>3.79</td>
<td>8.73</td>
<td>6.26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beef, mince, lean &amp; 15% tissue, fried</td>
<td>M544</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.4</td>
</tr>
<tr>
<td>Lamb, comp. cuts, lean, cooked</td>
<td>M301</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.47</td>
</tr>
<tr>
<td>Mutton, leg, lean, roasted</td>
<td>M598</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>779</td>
<td>4.4</td>
<td>18</td>
<td>6.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Venison, loin chop, fresh, raw</td>
<td>M186</td>
<td>...</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.82</td>
</tr>
</tbody>
</table>

Abbreviations: Fruit, ‘fruit and fruit juice’ food group; Inftcr, ‘iron-fortified infant cereal’ food group; Redme, ‘red meat’ food group; Vege, ‘vegetables’ food group
Table Appendix J. (continued) Examples of how some of the 1,682 individual foods (rows) were assigned ‘nutrient’ values for Energy (kJ), Iron (fe), Selenium (se), and Zinc (zn) (columns) for the analysis of the ‘nutrient’ contribution of foods to each food group (16 food groups)

| Descriptor                      | Food id | Fishpo_kj | Fishpo_se | Fishpo_zt | Otherme_kj | Otherme_se | Otherme_zt | Legum_kj | Legum_se | Legum_zt | Eggs_kj | Eggs_se | Eggs_sn |  |
|---------------------------------|---------|-----------|-----------|-----------|------------|------------|------------|----------|----------|----------|--------|--------|--------|  |
| Anchovy, canned in oil, drained | K181    | 100       | 812       | 2.5       | 90         | 2.0        | 0          | 0        | 0        | 0        | 0      | 0      | 0      | 0 |
| Chicken, breast, flash, grilled | M232    | 100       | 691       | 1.9       | 15.3       | 1.5        | 0          | 0        | 0        | 0        | 0      | 0      | 0      | 0 |
| Fish, battered, deep fried      | K22     | 100       | 1247      | 2.3       | 51.15      | 0.46       | 0          | 0        | 0        | 0        | 0      | 0      | 0      | 0 |
| Pork, schnitzel, lean, fat & skin, raw | M443 | 100 | 524 | 1.41 | 10.24 | 2.11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bacon, separable fat, grilled   | N37     | 100       | 100       | 0         | 0          | 0          | 100        | 2616     | 26       | 1.8      | 0      | 0      | 0      | 0 |
| Ham, sliced, premium           | N66     | 100       | 100       | 0         | 0          | 0          | 100        | 819      | 0.76     | 13.9     | 0      | 0      | 0      | 0 |
| Salmi, raw, fish                | N1008   | 100       | 100       | 0         | 0          | 0          | 100        | 997      | 2.7      | 20.1     | 0      | 0      | 0      | 0 |
| Sausage, ham and chicken luncheon | N10   | 100       | 100       | 0         | 0          | 0          | 914        | 0.95     | 11.44    | 1.66     | 0      | 0      | 0      | 0 |
| Beans, red kidney, boiled       | X141    | 100       | 100       | 0         | 0          | 0          | 0          | 0        | 0        | 0        | 0      | 0      | 0      | 0 |
| Hummus, original, & 5% fat, commercial | N73 | 100       | 100       | 0         | 0          | 0          | 535        | 1.58     | 5.55     | 122      | 0      | 0      | 0      | 0 |
| Lentil, split, boiled, drained  | X54     | 100       | 100       | 0         | 0          | 0          | 0          | 0        | 0        | 0        | 0      | 0      | 0      | 0 |
| Tofu, steamed, microwave        | E1034   | 100       | 100       | 0         | 0          | 0          | 0          | 0        | 294      | 2        | 0.25   | 0      | 0      | 0 |
| Egg, baked                      | R1429   | 100       | 100       | 0         | 0          | 0          | 0          | 0        | 0        | 0        | 100    | 0      | 0      | 0 |
| Egg, scrambled, plain          | R3958   | 100       | 100       | 0         | 0          | 0          | 0          | 0        | 0        | 0        | 0      | 0      | 0      | 0 |
| Egg, whole, raw                | G1008   | 100       | 100       | 0         | 0          | 0          | 0          | 0        | 0        | 0        | 100    | 530    | 17     | 23 |
| Omeletta, plain                | R63     | 100       | 100       | 0         | 0          | 0          | 0          | 0        | 0        | 0        | 100    | 712    | 1.45   | 19.54 | 0.83 |

Abbreviations: Eggs, ‘eggs’ food group; Fishpo, ‘fish and poultry’ food group; Legum, ‘legumes’ food group; Otherme, ‘other meat’ food group
Table Appendix J. (continued) Examples of how some of the 1,682 individual foods (rows) were assigned ‘nutrient’ values for Energy (kJ), Iron (fe), Selenium (se), and Zinc (zn) (columns) for the analysis of the ‘nutrient’ contribution of foods to each food group (16 food groups)

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Food id</th>
<th>Nutse_g</th>
<th>Nutse_kj</th>
<th>Nutse_fe</th>
<th>Nutse_se</th>
<th>Nutse_mz</th>
<th>Dairy_g</th>
<th>Dairy_kj</th>
<th>Dairy_fe</th>
<th>Dairy_se</th>
<th>Dairy_mz</th>
<th>Beve_g</th>
<th>Beve_kj</th>
<th>Beve_fe</th>
<th>Beve_se</th>
<th>Beve_mz</th>
<th>Misc_g</th>
<th>Misc_kj</th>
<th>Misc_fe</th>
<th>Misc_se</th>
<th>Misc_mz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almonds, dried, blanched</td>
<td>Q46</td>
<td>100</td>
<td>2470</td>
<td>3.63</td>
<td>1.6</td>
<td>3.16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hazelnuts, dry roasted, unsalted</td>
<td>Q39</td>
<td>100</td>
<td>2716</td>
<td>3.3</td>
<td>0</td>
<td>2.42</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peanut butter, smooth &amp; crunchy, no sugar, salt added</td>
<td>Q48</td>
<td>100</td>
<td>2357</td>
<td>1.4</td>
<td>10.95</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sunflower seeds, kernel, dried</td>
<td>Q41</td>
<td>100</td>
<td>2488</td>
<td>6.77</td>
<td>49</td>
<td>5.06</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Butter, unsalted, mainland</td>
<td>F1050</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>3106</td>
<td>0.05</td>
<td>1</td>
<td>0.08</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cheese, cheddar, mild</td>
<td>F1015</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>1766</td>
<td>0.05</td>
<td>8</td>
<td>3.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cream, sour, light</td>
<td>F100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>595</td>
<td>0</td>
<td>0</td>
<td>0.44</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ice cream, fruit ripple</td>
<td>F119</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>700</td>
<td>0.06</td>
<td>1</td>
<td>0.31</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Yoghurt, greek style</td>
<td>F1055</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>464</td>
<td>0.01</td>
<td>0.32</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drink Flavour, navel orange, diluted</td>
<td>CS7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>152</td>
<td>0.02</td>
<td>0</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Milk, soy milk- vitamin E calcium plus</td>
<td>R4742</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>244</td>
<td>0.44</td>
<td>1.4</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rice milk, rice dream, composite</td>
<td>C1008</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>127</td>
<td>0.04</td>
<td>0.1</td>
<td>0.08</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tea, herbal, ready to drink</td>
<td>C76</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alpice, ground</td>
<td>P121</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>954</td>
<td>73</td>
<td>17</td>
<td>1</td>
<td>405</td>
<td>100</td>
<td>75</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Cake, chocolate, butter icing</td>
<td>R34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>1569</td>
<td>21</td>
<td>12</td>
<td>1</td>
<td>121</td>
<td>100</td>
<td>1569</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Coconuts, cream, canned</td>
<td>Q26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>717</td>
<td>0.67</td>
<td>1</td>
<td>0.3</td>
<td>1.3</td>
<td>100</td>
<td>717</td>
<td>0.67</td>
<td>1</td>
</tr>
<tr>
<td>Dressing, oil &amp; mayonna</td>
<td>R3654</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>1801</td>
<td>0.45</td>
<td>1.2</td>
<td>0.07</td>
<td>0.07</td>
<td>100</td>
<td>1801</td>
<td>0.45</td>
<td>1.2</td>
</tr>
<tr>
<td>Honey</td>
<td>W11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>1336</td>
<td>0.43</td>
<td>1.8</td>
<td>0.14</td>
<td>0.14</td>
<td>100</td>
<td>1336</td>
<td>0.43</td>
<td>1.8</td>
</tr>
<tr>
<td>Oils, rice bran</td>
<td>J1024</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>3700</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>3700</td>
<td>0.07</td>
<td>0</td>
</tr>
<tr>
<td>Sauce, oyster</td>
<td>S63</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>530</td>
<td>3.58</td>
<td>0</td>
<td>0.09</td>
<td>0.09</td>
<td>100</td>
<td>530</td>
<td>3.58</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: Beve, ‘beverages’ food group; Dairy, ‘dairy’ food group; Misc, ‘miscellaneous’ food group; Nutse, ‘nuts and seeds’ food group
Appendix K: Protocol for arranging blood collection appointment
**Purpose**

**Objective:**
Call each participant at 11 months to arrange the blood collection time (when the 12-month measurement visit is being booked).

**Equipment required**

**Protocols:**
- P-52: Phone call to arrange blood collection time (this protocol)
- P-55: Frequently asked questions about blood collection

**BLISS database:**
Program open with participant details

**Blood test appointment sheet:**
Listing dates & times available for blood test (Lisa is coordinating this)

**Paperwork:**
Appointment card (ready to fill in)

**Steps - before**

1. Check – has the participant consented to the blood test?
2. Have the BLISS database open – find participant information.
3. Have the “Blood test appointment sheet” (listing dates and times when Glenna, Lisa and the Clinic are available) ready to use during the phone call.
4. Have an appointment card ready to send following the phone call.

**Arrange appointment – Telephone call**

1. Hi, this is [say name] calling from the BLISS study at the University of Otago. Is it OK for me to talk with you just now to arrange an appointment? It will take a couple of minutes.
2. I am calling to ask you if we can organise a time for [baby’s name] to have their 12-month blood test?
3. The blood test is to determine [baby’s name] iron and zinc status. This is very important information for the study because so many New Zealand toddlers are low in iron and we need to find ways of preventing this happening. We will also be able to tell you if [baby’s name] has anaemia so that they can be treated.

4. During the appointment an experienced health professional will take a small blood sample. We use a very effective anaesthetic gel that we hand out at the 12-month measurement visit for you to put on [baby’s name’s] arm 1 to 4 hours before the blood test.

5. The blood test appointment will take about 30 minutes and will take place at the Human Nutrition clinic at the University of Otago.

6. Do you have any questions?

   YES – Answer using P-55: Frequently asked questions about blood collection; If you don’t know the answer then ask them if they would like a researcher to call them.

   NO – Go to #7

7. Are you happy to arrange a time now?

   YES – Discuss available times (Record day/time on the “Blood test appointment sheet” and the BLISS database).

   NO – Call back to arrange a time.

8. We will give you an appointment card with directions for finding the clinic when you come to your 12-month measurement visit.

9. If you have any further questions before the blood collection appointment please call the BLISS Study Office. If we are not there – please leave a message on the answer phone and we will return your call as soon as possible.

---

**Steps - after**

1. Check the date and time for the blood collection appointment is recorded on the BLISS database.

2. Book the clinic room (G01) – [http://huntmrbs.otago.ac.nz/Rooms](http://huntmrbs.otago.ac.nz/Rooms)
   - Clinic room should be booked for 1 hour time slot and with the nurse.

3. Fill in the appointment card and put with participant’s file.
4. Task a reminder phone call – to complete illness questionnaire and remind about the blood test date and time.

5. Report any additional questions asked about the blood test and ask Anne-Louise to add the answer to **P-55: Frequently asked questions about blood collection.**
Clinic facilities booking

HOW TO BOOK RESEARCH/CLINIC FACILITIES ONLINE

- Go to http://huntmrbs.otago.ac.nz/Rooms or the Human Nutrition main page and click on the Quick Links and then the Clinic Booking System.

  Login: human nutrition
  Password: apple

- Using your cursor scroll over the date on the calendar you wish to make a booking. This should show you any current bookings.

- Click on the room and time you wish to book. Another window will open.

- Add the name of the study in the “Brief Description” box.

- In the “Full Description” box enter the following:
  - How many people are involved in the study
  - If special equipment is required that is only available in the room requested (ie. the treadmill).

- For the “Nurse/No Nurse” section, click Nurse if you require a Research Nurse and No Nurse if you do not. This will allow others to know quickly if a Research Nurse is needed for your study and book accordingly.

- Please remember to save your booking.

- Please remember when booking a room that because it is busy in the clinic at times Glenna/Andrea will need to confirm all bookings.

- If a participant cancels please notify Glenna/Andrea ASAP via email.

- Please do not book space more than two weeks in advance. It can be very busy and the research nurse is attempting to accommodate everyone.
Also, please do not book anything in the “Research Nurse” column as that room is only used at Glenna/Andrea’s discretion (e.g. booking meeting, workshops).

Glenna:
Cell Phone: 021 0772085
Email: midwife.glennapaterson@hotmail.co.nz

Clinic Phone: (03) 4791100 ext 7819
Blood test itself

1. **How much blood will you take?**
   About 7mL, which is about 1 and a ½ teaspoons

2. **Will it hurt?**
   - We give you a very effective local anaesthetic to put on baby’s arm before they come in.
   - We’ve tested it out on ourselves in a previous study and it worked very well.
   - We tested it by putting the local anaesthetic on one arm, and then having a blood test from each arm and comparing it – it doesn’t hurt at all having the blood test from the arm with the anaesthetic.

3. **Who will do the blood test?**
   A midwife who is a trained “phlebotomist” (i.e., has been trained in taking blood samples).

4. **How long will the appointment take?**
   About 30 minutes (the blood test itself is much quicker than this).

5. **Will it be distressing for my toddler?**
   - We need to hold baby’s arm still so that they don’t move while the blood test is happening
   - They can get pretty cranky about that
   - But the local anaesthetic means you can be reassured that the blood test doesn’t hurt them
   - And you can be there the whole time.

6. **What happens if my baby is sick for the blood test?**
   Please call us using the number on the back of the appointment card and we can re-schedule the appointment.

7. **Will there be any parking available?**
   We have some parks at the Human Nutrition Clinic at the University – we have some instructions on the appointment card we’ll give you at your measurement appointment.
Local anaesthetic

1. Will it hurt?
• We give you a very effective local anaesthetic to put on baby’s arm before they come in.
• We’ve tested it out on ourselves in a previous study and it worked very well.
• We tested it by putting the local anaesthetic on one arm, and then having a blood test from each arm and comparing it – it doesn’t hurt at all having the blood test from the arm with the anaesthetic.

2. How long will the numbness of the Ametop gel last for?
The numbness lasts between 4 and 6 hours after it is applied.

3. What should I do if my baby has a bad reaction to the Ametop gel?
Some side effects may occur, such as itching and swelling. Please contact your doctor or pharmacist only if these side effects worry you.

4. My child has eczema – will the anaesthetic be safe to use?
If both of baby’s arms are affected by eczema on the day then you shouldn’t put the gel on those areas. You can consider putting the gel put on the back of their hand instead.

Milk feed

1. Can I give my baby his/her cereal with the milk feed?
As we are measuring zinc we are aiming to standardise what the toddlers eat before the blood test. We ask that you only give your toddler milk before the blood test. You may feed him/her cereal first and then give the milk feed.

After the blood test

1. Will I get any results back from the blood test?
• We will be measuring iron status soon after the blood test is carried out, and will contact you if the results suggests your baby may have iron deficiency (usually within 10-14 days of the blood test).
• Because some of the things we measure (for example, haemoglobin) are affected by a range of health conditions – not just iron - sometimes something else will come up in the blood test.
• If the results are in any way outside the usual range, then we will contact you so you can contact your GP for advice.
2. **What happens if my baby has anaemia?**
We will send out a letter to you and your GP so that they can advise you on how to proceed.

### Why we are doing things the way we are

1. **Why do they need to have a milk drink 90 mins before the appointment?**
   - We are measuring zinc status (as well as iron status)
   - Zinc status is altered by having had food recently.
   - Milk is the one food we knew all toddlers were likely to have in their diet (breast milk, formula, or cow's milk) so we have asked everyone to offer some to their baby at the same time before the blood test.

2. **How common is iron deficiency in NZ toddlers?**
   A survey carried out by the Department of Human Nutrition suggests that up to 1 in 3 NZ toddlers may have suboptimal iron status (slightly more may have poor zinc status).

3. **Why are you worried about iron and zinc in toddlers?**
   - Suboptimal iron status can lead to iron deficiency anaemia
   - Iron deficiency anaemia can cause irritability, poor cognition (thinking ability) and developmental delay.
   - Zinc deficiency is associated with poorer growth, and lowered immunity.
   - Our earlier work in this age group suggested that NZ toddlers may be low in both iron and zinc.
   - The BLISS study is looking at whether different ways of introducing solids may be protective.

**ANYTHING COMPLEX – SAY YOU WILL ASK LISA TO CALL THEM TO ANSWER THEIR QUESTION**
Appendix L: Blood test appointment card
Blood Test Appointment Card

has an appointment at:

» Milk feed given at: ________________

» Ametop gel applied at: ____________

(Please place tube in bag provided and bring to the clinic)

Find us at:

Union Court Clinic G01
85 Union Place West
(Please use carparks 27, 28 or 29)

BLISS Study
Contact phone: (03) 471 6063 or 022 192 7421
Appendix M: Pre-blood test instruction sheet
Pre Blood Test - INSTRUCTION SHEET

Before your blood test appointment please follow these instructions:

1. Give your baby a milk feed and stop feeding exactly 90 minutes (1½ hours) before your blood collection appointment (they can have as much as they like of the milk they usually have, e.g., breast milk, formula, cows milk).
2. Please do not give them anything else to eat or drink (except water) until after their blood collection appointment.
3. Apply AMETOP Gel 1- 4 hours prior to your blood collection appointment (see instructions below).

How to use AMETOP Gel

DO NOT SWALLOW AMETOP GEL!

What is AMETOP Gel?
AMETOP Gel is a type of ointment known as a local anaesthetic. AMETOP Gel numbs the skin. It is used to relieve the pain when needles are inserted into a vein for taking a blood sample.

Before using AMETOP Gel
Is your child allergic to any of the items listed below:
- Tetracaine
- Sodium chloride
- Potassium phosphate
- Xanthan gum
- Sodium hydroxide
- Sodium methyl-p-hydroxybenzoate
- Sodium propyl-p-hydroxybenzoate

Is your child allergic to any other local anaesthetics?

If the answer to any of these questions is YES, you should NOT use AMETOP Gel and should inform the BLISS study office (phone: (03) 471 6063).
**Do not use AMETOP Gel on:**
- Wounds
- Broken skin (including skin affected by eczema (mild dryness or redness is OK))
- Lips, mouth or tongue
- Eyes or ears
- Anal or genital regions

If AMETOP Gel is swallowed, contact a Doctor IMMEDIATELY!

**How to use AMETOP Gel (It is a good idea to have another adult to help – call us if you’d like one of us to be there)**

1. To open the tube, use the top of the cap to pierce the seal.
2. Place half of the gel in a mound on the area of the skin to be numbed on the right arm (apply the gel to the region of the arm in the front of the elbow - see photo below). Apply the other half of the gel in a mound on the front of the left elbow. **DO NOT RUB THE GEL INTO THE SKIN.**
   
   **NB:** If both arms are affected by eczema in this region, then please place the gel on the centre of the back of the hand.

3. Place the **plastic film dressing** over the mound of gel (see instructions below). Smooth down the edges of the dressing right around the edges of the gel.
4. **Record the time** you applied the gel.
5. **Leave the gel on your child’s arm for at least 30 minutes, but not for longer than 1 hour.**
6. After 30-45 minutes, remove the plastic film dressing from the arm and wipe the gel off the skin with a clean tissue. Dispose of the used plastic film dressing and the tissue carefully, keeping out of reach of children.
7. Once you have used the AMETOP Gel, **place the tube in the plastic bag, which has your ID number on it.** Take this bag with you to your appointment at the clinic.
8. Go to the Human Nutrition clinic for the blood test within 4 hours of the gel being wiped off the skin (please see the map on your appointment card).

**Considerations**

Do not leave AMETOP Gel on your child's skin for more than 1 hour.

Take care to minimise contact with AMETOP Gel (except for the area of the skin to be numbed) during application or removal of AMETOP Gel.

**After using AMETOP Gel**

AMETOP Gel can cause side effects. Almost everyone gets some redness where the gel is applied, as the blood supply to the area is increased. This should pass in a few hours. Other side effects may include itching and swelling of the skin at the numbed site, or on very rare occasions, blistering. You need only tell your doctor or pharmacist if these effects worry you.
Storing AMETOP Gel
- Keep AMETOP Gel out of reach of children and pets.
- Use once. Take the used tube with you to your appointment (bag provided).
- Protect AMETOP Gel from heat. Do not freeze.
- The expiry date is printed on the base of the tube. Do not use AMETOP Gel after this date.

How to use plastic film dressing

1. Ensure skin around gel site is dry.
2. Remove dressing from pouch.
3. Remove backing paper marked 1, taking care not to touch the adhesive side.
4. Place the dressing over the gel site using light pressure.
5. Remove the backing paper marked 2 and press around the edges of the dressing.
6. Remove the plastic green grid marked 3.
7. To remove the dressing, lift one corner and slowly stretch the film in a motion that is parallel to the skin. Dispose of the used dressing immediately.

If you have any questions regarding AMETOP Gel, please contact us at:
BLISS Office
Phone: (03) 471 6063 or 022 192 7421
Email: bliss@otago.ac.nz
Appendix N: Protocol for reminder blood test phone call and illness questionnaire
Purpose

Objective: Call each participant 24 hours prior to blood collection appointment to remind them of the time; find out if any illness is present; remind them about the Ametop gel application and milk feed.

Equipment required

Protocols:  
- **P-53: Phone call reminder about blood collection** (this protocol)  
- **P-52: Phone call to arrange blood collection time** (in case need to rebook)  
- **P-55: Frequently asked questions about blood collection**

**BLISS database:** Program open with participant details

**Blood test appointment sheet:** Listing dates & times available for blood test (Lisa is coordinating this)

**Paperwork:** Printed copy of Pre Blood Test Instruction Sheet – own reference  
Printed copy of Illness questionnaire

Steps - before

5. Have the BLISS database open – find participant information.

6. Record participant ID number on Illness questionnaire.

7. Calculate the following times before the appointment time:  
   - 90mins (for milk feed)  
   - 1-4 hours (to apply Ametop gel)
10. Hi, this is [say name] calling from the BLISS study at the University of Otago. Do you have 5 minutes to talk about [baby’s name] blood test appointment tomorrow?

   YES – Continue
   NO – When would I be able to call back?

11. I would like to confirm that [baby’s name] appointment tomorrow at [time] at the Human Nutrition clinic still works for you?

   YES – Continue
   NO – Follow P-52: Phone call to arrange blood collection time. Thank and finish call.

12. Did you receive your appointment card at your measurement visit?

   YES – Carry on
   NO – Check they are happy with current appointment time and following Pre Blood Test Instruction Sheet. Otherwise, make a new appointment and send out a new appointment card.

13. Are you OK with finding the location for the blood test?

   YES – Carry on
   NO – There is a map on the appointment card to direct you to the clinic, which is at the University of Otago on Union Street. Answer questions.

14. Complete the Illness questionnaire.

15. Have you had a chance to read through the Pre Blood Test Instruction Sheet? [The participant should have received this information at the 12-month visit].

   YES – Go to Question 8
   NO – Go to Question 7

16. Did you receive a copy of it at [baby’s name] 12-month visit?

   YES – That’s great – would you like me to go over the instructions briefly now?
   NO – I’m sorry about that. Did you get a small ziplock plastic bag with the Ametop gel in it? I will send you out the [Information Sheet/Blood Test Information Kit] in the mail. Unfortunately, we will need to reschedule your appointment to allow time for this to arrive. Can we
make a new appointment time now? Follow P-52: Phone call to arrange blood collection time. Thank and finish call.

17. Do you have any questions about applying the Ametop gel?

YES – Answer using P-55: Frequently asked questions about blood collection

NO – Carry on

18. Also, just to remind you if you could please:

(a) Give [baby’s name] a milk feed (as much as s/he likes of whichever milk s/he normally has) at [time – calculate at what time they need to do this (appt time – 90 mins)]

(b) Then no other food or drink (except water) until after the appointment

(c) Apply the Ametop gel between [time – 4 hrs prior to blood test] and [time – 1 hr prior to blood test]

[you may want to suggest giving the milk feed at 1½ hrs prior to the blood test and then applying the gel straight after the feed].

19. Do you have any other questions about this appointment?

YES – Answer using P-55: Frequently asked questions about blood collection

NO – Carry on

20. Thank you for your time. I look forward to seeing you and [baby’s name] tomorrow at [time] at the Human Nutrition clinic.

Steps - after

1. Check the date and time for the blood collection appointment is recorded on the BLISS database.
   - If appointment needs to be rescheduled due to illness, arrange to call participant in 1 week’s time, then follow P-52: Phone call to arrange blood collection time.

   - If appointment needed to be rescheduled due to missing Pre Blood Test Information Sheet or Blood Test Information Kit, arrange for information /kit to be sent to the participant.

2. File the completed Illness questionnaire.

3. Report any additional questions asked about the blood test and ask Anne-Louise to add the answer to P-55: Frequently asked questions about blood collection.
Pre blood test - Illness Questionnaire

*If baby has had a temperature of greater than 37.5 degrees C in the past 24 hours, and/or has “been unwell” in the past 2 weeks (including symptoms of diarrhoea and/or vomiting, do not count teething, or “just not herself”) the blood test appointment should be re-scheduled for 2 weeks time.*

1. Has baby had a fever in the past 24 hours?
   - Ø YES – Go to Question 2
   - Ø NO – Go to Question 4

2. What was baby’s highest temperature reading? *(A child is considered to have a fever when his or her oral temperature is higher than 37.5 degrees C (National Institute of Health, 2013)).*

3. How was this temperature measured?
   - Ø Mouth
   - Ø Ear
   - Ø Armpit
   - Ø Other Please state: __________

4. Has baby “been unwell” in the past 2 weeks? Please do not count “teething”.
   - Ø YES – Go to Question 5
   - Ø NO – Thank you – you have finished the questionnaire

5. What was baby unwell with?

________________________________________________________________________

Symptoms: __________________________________________________________________

Period of illness: __________________ Treatment: Ø Yes Ø No

Period of treatment: ___________ Type of treatment: __________

Appendix O: Protocol for blood sample collection and analysis
P-54: Pre and post blood collection

Purpose

Collect data: Blood sample (7.5mL Sarstedt tube)
Blood collection checklist
Zinc questionnaire

Process blood samples: 7.5mL Sarstedt tube for determining iron and zinc status
Identify abnormal results and communicate these results with participant and participant's GP

Equipment needed

Protocols: P-54: Pre and post blood collection (this protocol)

Paperwork: Blood collection checklist
Zinc questionnaire
Biochemical sample inventory

Equipment: BLISS labelled chilly bin (Appendix 1)
2 x frozen freezer blocks (stored in the BLISS kitchen freezer)
Polyestrene tray to sit Sarstedt tube into
7.5mL trace-element free Sarstedt tube x 2
- 1 labelled with details
Mulifly needle x 3
Gift
Toys for distraction

Southern Commuity Laboratories (SCL) test tube x 1
- labelled with details
Trace-element free transfer pipettes x 6
Microcentrifuge (MCT) tubes x 4
- 3 labelled with details
Marker pens for labelling tubes for blood disposal
- Red = Karakia
- Green = Return to participant

Other: Researcher name badge
Ordering Equipment

**Frances (Accounts):**
Sarstedt Products
1. S-Monovette Trace Element Metal 7.5mL 92X15MM - SARS01.1640.400 (box of 50)
2. Multiply Needles - SARS85.1640.005_U (box of 100)
NB: Check expiry dates before ordering.

**Roger Barton (SCL):**
Test tubes with lids

---

**Steps - before**

**P-29: 12 month BLISS visit protocol** has been completed (i.e. AMETOP Gel and milk feed instructions delivered).

**Day before:**
1. Find out when the participant is arriving – check the BLISS database and confirm time with phlebotomist (Glenna).

2. **P-53: Phone call reminder about blood collection** to be completed.

3. Familiarise yourself with this protocol.

4. Prepare all materials needed on equipment list, including labels on blood collection tube, SCL tube and MCT tubes (Appendix 1).
   a. Label blood collection tube (Sarstedt) using sticker label with participant ID and date.
   b. Label SCL tube with participant ID sticker only.
   c. Label 3 x MCT tubes with one of each sticker labelled with participant ID:
      i. Zn+Se
      ii. A1GP, CRP, STR
      iii. Spare
   d. Label the top of the tubes with participant ID, using permanent marker.

5. Wrap sellotape around the labels on each tube. Make sure that the sellotape covers the stickers well, this is to prevent the text on the stickers from rubbing off and the sticker from falling off the tube. Mark any tubes without stickers with a permanent marker.

6. Label tubes (with a permanent marker) with samples to be returned to participant with a green dot and Karakia samples labelled with a red dot (label top and side of tubes). All other samples should be unmarked.
7. Ensure all paperwork is labelled with the correct participant ID.

8. Contact Karl to let him know you will be bringing samples into the lab - 021 170 6057.

**On the day:**
1. Arrive 15 minutes before participant is due to arrive at the clinic.

2. Check you have all the required paperwork and equipment (including freezer blocks from freezer) and put on researcher name badge.

3. Make sure that the phlebotomist is familiar with the section below on “General principles for phlebotomy in BLISS”.

<table>
<thead>
<tr>
<th><strong>Steps - during</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reintroduce/introduce self and convey our thanks for taking the time to see us today.</td>
</tr>
<tr>
<td>2. Welcome the participant into the clinic – ensure the participant has found the correct location for parking.</td>
</tr>
<tr>
<td>3. Always try to use the baby’s name to personalise the messages.</td>
</tr>
<tr>
<td>4. Check the identity of the participant and go through the blood collection checklist.</td>
</tr>
</tbody>
</table>
| 5. Explain what is going to happen during the appointment  
  a. During this appointment [phlebotomist] will be taking a small blood sample from [baby’s name].  
  b. We will collect some information about the milk feed and how things went with the Ametop gel.  
  c. We also have a very short questionnaire.  
  d. The appointment should take no longer than 30 minutes. |
| 6. Fill out the zinc questionnaire. |
| 7. Ask about when the Ametop gel was applied and removed – the gel needs to have been on for at least 30 minutes, no more than 4-6 hours ago for it to work. |
8. Introduce the participant to the phlebotomist who will give an explanation of the procedure and complete the blood sample collection (see “General principles for phlebotomy in BLISS” below).

9. Help to position toddler and distract if need be, also be there to reassure parent. Hold arm firmly but kindly.

10. Check to make sure tourniquet time is minimised – 1 minute use only.

11. Place blood samples in the cooler container – keep sample directly off ice, sit in tube holder.

12. Check everything is ticked off on the blood collection checklist.

13. Thank the participant for their time (gift given to child) and let them know that the next visit will be at either 12 or 24 months depending if the 12 month appointment has been completed prior to the blood test.
General principles for phlebotomy in BLISS

- Ametop gel should have been applied to two areas – usually both antecubital regions (sometimes the back of the hand).
- Only attempt blood testing a maximum of once in each anaesthetised region.
- Minimise tourniquet time – 1 minute use only.
- If some blood has been collected but then the vein collapses, or flow stops for some other reason, then do not make another attempt - record what happened on the checklist sheet.
- Usual arrangement is to have the toddler seated on the parent’s lap with the parent's arms wrapped around the toddler’s torso. One arm will be stretched out and held firmly by a BLISS research assistant.
- Wherever possible, do not have the next participant waiting outside the room while another toddler is having their blood test - they get pretty cranky at being held still and this can be misconstrued by others waiting in the waiting area.
- Ideally a blood sample of 5-6 ml will be collected, but the following minimum amounts are still useful:
  - 2ml allows haemoglobin, plasma ferritin and zinc measurement
  - 1ml allows haemoglobin and plasma ferritin measurement
  - 0.5ml allows haemoglobin measurement
- Distraction with a toy can be very helpful!
- Keep any food away from the blood collection area and any blood samples.
Steps after - 1. Storage and processing of blood samples

Clinic:

1. Any blood samples should be kept on ice (without the ice touching the tube) in the clinic until taken to the lab.

Lab (7C9):

1. Contact Karl either the day before or on the day to let him know you will be bringing up some blood samples. 
   Karl = 021 170 6057

2. For samples of at least 4mL:
   a. Pipette 1.2mL or 1mL minimum (for smaller samples) whole blood into labelled SCL tube.
   b. Spin whole blood at 2000-3000 x g (3500 rpm) for 10 minutes. Note on blood collection checklist the time of separation (start of spin).
   c. Aliquot plasma into 3 x labelled MCT tubes (ensure each tube is labelled with correct disposal options).
      i. Zinc + Selenium = 0.7mL
      ii. α1-acid glycoprotein, C-reactive protein, Soluble transferrin receptor = 0.5mL
      iii. Spare = remaining plasma

3. For smaller samples, prioritise:

   1. CBC + ferritin
   2. Inflammatory markers (AGP, CRP and soluble transferrin receptor - all done together)
   3. Zinc
   4. Selenium
   5. Spare

4. Bring tubes to Karl’s -80°C freezer (6s 14).

5. Store samples in trays with covers. Write down the coordinates of where the sample is located on the biochemical inventory sheet (box number, corresponding letter and number coordinate).
6. Freeze remaining red cells, in microcentrifuge tubes labelled with participants ID in Karl's -80°C freezer (6s14).

7. Return freezer blocks to metabolic kitchen freezer.

**Southern Community Lab Work:**

1. Make sure the SCL tube is labelled with study name and participant ID.

2. Take SCL tube to Southern Community Labs - 3rd floor clinical services building – Cumberland Street (carry in chilly bin, labelled with study name and biohazard warning).

3. Leave the labelled SCL tube/s with either Roger or Vivienne, if neither are there, then ask to leave sample for Roger or Vivienne.

4. Pick up analysed sample:
   a. Dispose of plasma in requested manner (standard/karakia/return). Dispose of standard method samples in any of the yellow biohazard bins in the union court clinic. Bring Karakia samples up to Karl's lab 7C9 and place in fridge with other karakia samples.
   b. Pipette remaining red cells into 2 x MCT tubes labelled with participant ID and date of blood collection.
   c. Place labelled tubes in labelled red cells box in Karl's -80°C freezer (6s14).

*Notes:*
- A maximum of 600 microcentrifuge tubes of blood will be stored.
- Whole blood CBC and plasma ferritin will be analysed at Southern Community Labs on the same day that blood is collected.

---

**Steps after – 2. Filing of blood-paper work**

File all **Blood Collection Checklists** in locked filing cabinet.
Steps after – 3. Results

- When results come in – identify abnormal results (*P-56: Communicating abnormal laboratory results*).

- When results appear to be abnormal use protocol *P-56: Communicating abnormal laboratory results* and prepare a letter to be sent to the participants GP (with results), if consented for this and to the parent (without results).
Appendix 1

Photographs of supplies:

Figure 8. Collection tubes

Figure 9. Chilly bin for transporting blood samples
Figure 10. Gifts
Appendix P: Checklist for during blood collection appointment
Blood Collection Checklist

1. Check participant name and DOB match  ○

2. Was the Ametop gel applied prior to the blood test?
   - Yes  ○  Time: __________
   - No  ○  Why not?: __________

3. Collected Ametop gel tube?
   - Yes  ○  If Yes, used  ○  or unused  ○
   - No  ○

4. What is the name of baby’s GP? *(In case the blood test results are outside the usual range)*

   ________________________________

5. How would they like their child’s biological sample disposed of (please circle one):
   - a) standard methods   - b) with karakia (prayer)   - c) returned to me

6. Has the zinc questionnaire been administered with participant ID labelled?
   - Yes  ○
   - No  ○

7. Date of blood collection:  8. Time of blood collection:
   ————  AM  ————  PM

Office use only

Date:
Code:
9. Was a blood sample obtained from the toddler?

<table>
<thead>
<tr>
<th>Sarstedt tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes ☐ No ☐</td>
</tr>
<tr>
<td>Why not? ____________________</td>
</tr>
</tbody>
</table>

10. How many attempts were made ____________ and what was the location of
    the blood draw ____________

11. Is the Sarstedt tube clearly and correctly labelled?

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

12. Child has been given a book?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

### Blood Processing Checklist

1. Time of blood separation: ____________

2. Time between blood sample taken and separation: ____________

3. Centrifuged sample at ________ (speed) for ________ minutes.
Appendix Q: Zinc questionnaire for during blood collection appointment
Zinc Questionnaire

1. What time did your child last eat food or drink milk prior to the blood collection?

   [ ] AM
   [ ] PM
   What did they eat/drink and how much?
   Meal [ ] ____________
   Milk feed [ ] ____________

2. Has your child had a fever in the past 24hrs?
   [ ] Yes
   [ ] No

3. Has your child had any other illness in the past 2 weeks?
   [ ] Yes
   Describe __________ How long ago did they recover? __________
   [ ] No

4. Have you used any of the following preparations on your child in the past month? (see illustrations on attached page)

   Zinc ointment [ ] Brand: ____________
   Eczema treatment cream [ ] Brand: ____________
   Nappy rash ointment [ ] Brand: ____________

NB: Do NOT include:
- Bepanthen and Bepanthen ointment
- Lucas pawpaw – ointment
- Vaseline
- Johnson’s original baby powder
- Johnson’s Desitin – multi-purpose ointment
Common Zinc Containing Products
Appendix R: Protocol for communicating abnormal blood results
Purpose

Objectives: Identify abnormal results. Communicate these results to the participant and participant's GP.

Steps

1. Detect abnormal results as they are received from Southern Community Labs (SCL).
   - Any one or more of the following results outside the normal reference range:
     - Plasma ferritin 15-150 μg/L
     - Haemoglobin 105-140 g/L
     - Hematocrit (packed cell volume) 0.32-0.41
     - Mean cell volume 70-86 fL
     - Mean cell haemoglobin 23-30 pg

2. Identified abnormal results need a letter sent to the participant and faxed and sent to their General Practitioner (GP) (contact details of GP collected on consent form and at time of blood test).
   - If no consent is given to send abnormal results to the GP, then an alternative should be arranged with the participant.

3. Prepare letters for the participant and GP using the letter templates.
   - Dropbox>BLISS SHARED>Protocols>Bloods>Abnormal results
   - Letter to GP template (see Letter to GP)
   - Letter to participant template (see Letter to participant)

4. Print the completed letters, check them and then copy onto University of Otago letterhead.

5. Call the requested medical centre and check the fax number to send the results to.
   - Look online for the medical centres contact phone number and fax number.
   - Call the medical centre and talk to the receptionist.
   - Explain that we are conducting a study at the University of Otago and that some results have been taken from a patient at the medical
centre and we would like to send the results through to the patients requested GP.
d. Check with the receptionist that the fax number is correct.
e. Explain that the results will be faxed and then the hard copy will be sent in the mail.

6. Go through checklist (see over the page) to ensure details are complete.

7. Fax letter and results to GP clinic.

To fax:
   a. Go to 7th floor Human Nutrition office
   b. Ask Anne to use fax machine
   c. Dial 1 and then dial the fax number
   d. Wait for copy page to return with status - OK

8. Send the hard copy of the letter along with a copy of the results and Information Brochure for Parents/Guardians to the requested GP.
   a. Look online for the mailing address of the medical centre.
   b. When addressing the envelope ensure it is directed to the relevant GP.
      [Name of medical centre]
      Attn: Dr [last name]
      [address]

9. Send the hard copy of the letter to the participant.
   a. Find participants contact details on the BLISS database.

10. File copies away with other blood test information in the locked filing cabinet (room 7s9 – bottom draw under the telephone).

**Checklist for sending letters**

- GP name and address is correct on letter
- Correct names (see letter template) entered into letter templates
- Correct participant DOB on letter to GP
- Letters printed
- Hard copy of letters proof read
- Copied onto University of Otago letterhead paper
- Signed
- Laboratory results printed
- Non requested laboratory results crossed out
- Fax letter and results to GP – received status OK
- Hard copy of letter and results sent to GP with Participant Information Brochure
- Hard copy of letter sent to participant
- Hard copies filed in locked filing cabinet (room 7s9)
Dear Dr [name],

Re: [Child participant name] [(DOB --/--/-----)] child of [Parent participant name]

The University of Otago is currently undertaking a study called the BLISS Study (the Baby-Led Introduction to SolidS study) to investigate the impact of a modified version of Baby-Led Weaning on iron and zinc status in toddlers. Please find enclosed our Information Brochure for Parents/Guardians, for your information.

Your patient, [Child participant name], provided a blood sample for the study on [--/--/-----], which was analysed by Southern Community Laboratories. The results of the blood test for complete blood count and plasma ferritin are enclosed. [His/her] parents have been advised that one or more of the results were outside the expected range and to contact you to discuss the results. Because the blood sample was collected into a Lithium-heparin anticoagulated vacutainer (to prevent trace element free contamination of the sample for zinc analysis), only the core complete blood count indices should be used. We have crossed out the results that will have been impacted by the anticoagulant.

Should you require any further information, please contact me at the University of Otago, phone (03) 479 7948.

Yours sincerely,

Lisa Daniels
PhD Candidate
BLISS Study
Department of Human Nutrition
Phone (03) 479 7948

Anne-Louise Heath, PhD
Co-Principal Investigator
BLISS Study
Department of Human Nutrition
Dear [Parent and child participant names],

Thank you very much for your participation in the BLISS study and for taking part in the blood test component of the study. We have now received the results back from this, and although most of the results from the test were as expected, one or more of the values were outside the expected reference range. This is not uncommon in this age group but we advise you to contact your General Practitioner to discuss the results. We have sent a copy of the results to your doctor, Dr [name].

If you would like to discuss anything to do with the blood test or the BLISS study as a whole, please feel free to call us (contact details below). Thank you again for taking part in the BLISS study.

Yours sincerely,

Lisa Daniels
PhD Candidate
BLISS Study
Department of Human Nutrition
University of Otago
Phone (03) 479 7948

Dr Anne-Louise Heath
Co-Principal Investigator
BLISS Study
Department of Human Nutrition
University of Otago
Phone (03) 479 8379

BLISS Study Office
Phone (03) 471 6063 or 022 192 7421
bliss@otago.ac.nz
Appendix S: Analysis of methodological factors known to affect plasma zinc concentrations
Table Appendix S. Methodological factors that are known to affect plasma zinc concentration \(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Total (n=115)</th>
<th>Control (n=57)</th>
<th>BLISS (n=58)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of sampling</td>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>0800-0900</td>
<td>11 (10)</td>
<td>4 (7)</td>
<td>7 (12)</td>
<td></td>
</tr>
<tr>
<td>0900-1000</td>
<td>56 (49)</td>
<td>32 (56)</td>
<td>24 (42)</td>
<td></td>
</tr>
<tr>
<td>1000-1100</td>
<td>36 (31)</td>
<td>15 (26)</td>
<td>21 (36)</td>
<td></td>
</tr>
<tr>
<td>1100-1130</td>
<td>12 (10)</td>
<td>6 (11)</td>
<td>6 (10)</td>
<td></td>
</tr>
<tr>
<td>Time since last meal</td>
<td></td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>&lt;90 minutes</td>
<td>11 (9)</td>
<td>5 (9)</td>
<td>6 (10)</td>
<td></td>
</tr>
<tr>
<td>90-120 minutes</td>
<td>70 (61)</td>
<td>36 (63)</td>
<td>34 (59)</td>
<td></td>
</tr>
<tr>
<td>&gt;120 minutes</td>
<td>34 (30)</td>
<td>16 (28)</td>
<td>18 (31)</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Summer</td>
<td>31 (27)</td>
<td>18 (32)</td>
<td>13 (23)</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>35 (30)</td>
<td>17 (30)</td>
<td>18 (31)</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>21 (18)</td>
<td>11 (19)</td>
<td>10 (17)</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>28 (25)</td>
<td>11 (19)</td>
<td>17 (29)</td>
<td></td>
</tr>
<tr>
<td>Acute phase protein status (^b)</td>
<td></td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Normal</td>
<td>96 (83)</td>
<td>49 (86)</td>
<td>47 (81)</td>
<td></td>
</tr>
<tr>
<td>Incubation</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Early convalescence</td>
<td>2 (2)</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Late convalescence</td>
<td>17 (15)</td>
<td>6 (11)</td>
<td>11 (19)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Values are n (%)

\(^b\)Normal: CRP <5 mg/L and AGP <1 g/L, Incubation: CRP >5 mg/L and AGP <1 g/L, Early convalescence: CRP >5 mg/L and AGP >1 g/L, Late convalescence: AGP >1 g/L and CRP <5 mg/L