

Doctoral Thesis for the degree of Doctor of Philosophy, Faculty of Food Science and  
Nutrition

# **The effects of diet during the first six months of life on health outcomes in infancy and early childhood**

**Ólöf Helga Jónsdóttir**



**UNIVERSITY OF ICELAND**  
**SCHOOL OF HEALTH SCIENCES**

University of Iceland  
Faculty of Food Science and Nutrition  
School of Health Science  
Reykjavik 2013



*Academic Dissertation*

University of Iceland, Faculty of Food Science and Nutrition, School of Health Science and  
Landspítali - The National University Hospital, Unit for Nutrition Research

*Supervised by*

Professor Inga Thorsdóttir, University of Iceland

*PhD Committee*

Professor Inga Þórsdóttir, University of Iceland

Dr. Geir Gunnlaugsson, Chief Medical Officer for Iceland and Professor at Reykjavík  
University, Iceland

Professor Jonathan C. Wells, Childhood Nutrition Research Centre, UCL Institute of Child  
Health, UK

Professor Patricia L. Hibberd, Division of Global Health, Massachusetts General Hospital for  
Children, Harvard Medical School, USA

Professor Ronald E. Kleinman, Department of Pediatrics, Massachusetts General Hospital for  
Children, Harvard Medical School, USA.

*Opponents*

Professor Anna Birna Almarsdóttir, University of Iceland

Dr. Elisabet Kylberg, Uppsala University and University of Skövde, Sweden



Dissertation for the Degree of Ph.D. in Nutrition

©Ólöf Helga Jónsdóttir, 2013

Design: Prentlausnir

Printed in Iceland by Prentlausnir, Reykjavík 2013

ISBN 978-9935-9130-1-2

*Maður uppsker eins og maður sáir.*

## Abstract

**Background:** The immediate and long-term benefits of breastfeeding for both mother and infant are well documented, but less is known about the impact of exclusive breastfeeding on health outcomes in infancy and childhood. In 2001 the World Health Organization (WHO) changed the recommended duration of exclusive breastfeeding from the first 4-6 months to the first 6 months of life. The principle reason for this change was to provide optimal nutrition to young infants in low resource countries where available water and complementary foods may be nutritionally inadequate or contaminated. In resource rich countries, the evidence for recommending 6 months of exclusive breastfeeding is less clear, nor are the nutritional consequences to the infant of this recommendation well-tested. Furthermore, recognizing that few randomized controlled trials contribute to the evidence base of the recommendations, the WHO requested such trials to guide future policy decisions in this regard.

The principle aim of the present thesis was to enhance our understanding of the effects of diet during the first 6 months of life on health outcomes in infancy and early childhood. Impact of lactation consultants vs. traditional consult in the well-baby clinics on the diet given to infants during the initial complementary feeding period as well as the impact of exclusive breastfeeding for 6 months on total breastfeeding duration were also studied.

**Methods:** 119 mother-infant pairs from 7 healthcare centers in Iceland were randomly assigned to receive complementary foods from the age of 4 months in addition to breast milk (CF), or to continue exclusive breastfeeding to the age of 6 months (EBF). Inclusion criteria in the study for infants were: singleton birth, gestational length  $\geq 37$  weeks, exclusively breastfed at the time of assessment and infant characterized as healthy. Furthermore, mothers had to be willing to be randomized into either intervention group. Baseline information was collected on eligible mother-infant pairs. Data were collected on complementary feeding intake at 5.25 months, breast milk intake from 5.5 to 6 months, iron status and infant body composition at 6 months, anthropometric outcomes from birth to 29-38 months of age, developmental and behavioral outcomes at both 18 and 30-35 months of age and total breastfeeding duration.

To be able to test the impact of lactation consultants on the diet given to infant's data were also taken from an additional cohort of infants born between January and December 2005 and randomly selected by Statistics Iceland for a separate national prospective study conducted in Iceland. Inclusion criteria for participation in the study were singleton birth, gestational length at least 37 weeks and absence of congenital abnormalities or chronic health issues. Infants' dietary intake data was collected prospectively, monthly from 5-8 months of age by 24-hour food recall. Information on total duration of breastfeeding was also collected.

**Results:** Infants in the EBF group consumed significantly more breast milk at 6 months ( $901 \pm 158$  g/d) compared with those in the CF group ( $818 \pm 166$  g/d;  $p=0.012$ ). Adjustment for characteristics that were significantly different at baseline did not change the findings. The average energy intake from complementary foods by infants in the CF group was  $265.4 \pm 219.0$

kJ/d ( $63.4 \pm 52.3$  kcal/d) or 36.8 kJ/kg body weight (8.8 kcal/kg); only 10% of the average daily energy requirements for infants 6-11 months of age. Infants in the CF group had higher mean serum ferritin levels at 6 months of age ( $p=0.02$ ) that remained significant when adjusted for baseline differences. No difference was seen between groups in the prevalence of iron deficiency anemia, iron deficiency or iron depletion. Furthermore, no difference was seen between groups in growth between 4 and 6 months or infants' body composition at 6 months.

The follow-up showed that duration of exclusive breastfeeding for 4 vs. 6 months did not affect developmental or behavioral outcomes at 18 months or 30-35 months of age. However, at 30-35 months of age a significantly smaller percentage of parents in group CF (2%) had concerns about their children's gross motor development compared to those in group EBF (19%;  $p=0.01$ ), which remained significant when adjusted for differences in pre-randomization characteristics ( $p=0.03$ ). Furthermore, the introduction of complementary foods at 4 months did not affect the prevalence of overweight children at 18 months ( $p=0.74$ ) or 29-38 months of age ( $p=0.36$ ).

In the analysis conducted with the additional cohort from the national prospective study, having unlimited access to lactation consultants appeared to support a slower introduction of complementary foods during the initial complementary feeding period, measured by the number of daily meals consumed by the infants at 6 months ( $p=0.001$ ). Furthermore, among all mothers with unlimited access to lactation consultants, those who introduced complementary foods at 6 months had a longer duration of total breastfeeding ( $1.08 \pm 0.15$ ) than those with infants receiving complementary foods from 4 months of age ( $1.01 \pm 0.11$ , 95% CI -0.001, 0.16) and those whose mothers had only routine care instead of unlimited lactation consultations at the well-baby clinics ( $0.96 \pm 0.13$ , 95% CI 0.03, 0.22). All infants exclusively breastfed for 6 months had similar duration of total breastfeeding, regardless of exposure to lactation consultants.

**Conclusion:** In a resource rich country, exclusive breastfeeding for 6 months does not compromise body composition or growth in infancy, because of similar energy intakes in both intervention groups. Adding a small amount of complementary foods in addition to breast milk to infants' diets from 4 months has a small and positive effect on iron status at 6 months. The biological importance of this finding remains to be determined. Breastfeeding support may slow the introduction of complementary foods and exclusive breastfeeding for 6 months may enhance more prolonged breastfeeding.

No sustained effect of a longer duration of exclusive breastfeeding was seen on selected measures of developmental and behavioral status at 18 months, although at 30-35 months, a smaller percentage of parents of children introduced to complementary foods at 4 months of age expressed concerns about their gross motor development. Furthermore, no effects of exclusive breastfeeding for 4 or 6 months were seen on the risk of being overweight or obese in early childhood. Our results underline the importance for larger randomized controlled trial on optimal duration of exclusive breastfeeding.

**Keywords:** Exclusive breastfeeding, complementary feeding, randomized trial, breast milk, iron status, growth rate, developmental status, lactation consultant, total breastfeeding duration.

## Ágrip

**Inngangur:** Jákvæð áhrif brjóstamjólkur eru vel þekkt, bæði fyrir móður og barn, en minna er vitað um áhrif þess að barn nærast eingöngu á brjóstamjólk, undanskilinn annar vökvi og föst fæða, á heilsu þeirra á ungbarnaskeiði og í barnæsku. Frá árinu 2001 hefur Alþjóðaheilbrigðismálastofnunin ráðlagt að börn séu eingöngu á brjósti til 6 mánaða aldurs, en áður fyrr var ráðlagt að börn fengju eingöngu brjóstamjólk í 4-6 mánuði. Ráðleggingunum var breytt aðallega á grunni ávinnings af eingöngu brjóstamjólki í 6 mánuði á tíðni meltingarfærasjúkdóma, þá sérstaklega í þróunarlöndunum þar sem vatn og viðbótarfæði getur verið mengað eða ekki nógu næringarríkt. Í iðnríkjunum eru vísindalegar sannanir fyrir því að ráðleggja eingöngu brjóstamjólki í 6 mánuði ekki eins augljósar. Á sama tíma og ráðleggingunum var breytt kallaði Alþjóðaheilbrigðismálastofnunin eftir slembiröðuðum íhlutandi rannsóknum á þessu sviði til að ganga úr skugga um að eingöngu brjóstamjólki í 6 mánuði hafi engar neikvæðar heilsufarslegar afleiðingar fyrir barnið.

Meginmarkmið þessarar rannsóknar var að auka þekkingu á næringu ungbarna fyrstu 6 mánuði ævinnar, sem og á vexti, járnþúskap og öðrum heilsufarsbreytum á ungbarnaskeiði og í barnæsku. Einnig voru rannsökuð áhrif brjóstagjafaráðgjafa og hefðbundinnar ráðgjafar í ung- og smábarnavernd á innleiðingu viðbótarfæðis snemma á ungbarnaskeiði, sem og áhrif eingöngu brjóstagjafar fyrstu 6 mánuði ævinnar á heildarlengd brjóstagjafar voru könnuð.

**Aðferðir:** 119 mæðrum og börnum þeirra frá 7 heilsugæslustöðvum á Íslandi var slembiraðað í hóp barna sem samhliða brjóstagjöf fengu aðra fæðu við 4 mánaða aldur (CF) eða þeirra sem fengu eingöngu brjóstamjólki til 6 mánaða aldurs (EBF). Skilyrði fyrir þátttöku í rannsókninni voru: einburi, a.m.k. 37 vikna meðganga, eingöngu brjóstamjólki við 4 mánaða aldur barns og að barn væri heilbriggt og án heilbrigðisvanda. Mæður þurftu einnig að vera tilbúnar að slembiraðast í báða íhlutunarhópana. Við upphaf rannsóknarinnar voru skráðar vissar grunnupplýsingar um barnið og fjölskylduna en að öðru leyti var stuðst við upplýsingar sem skráðar voru í heilsufarsskrá barnsins. Gögnum var safnað um inntöku viðbótarfæðis við 5.25 mánaða aldur, inntöku brjóstamjólkur frá 5.5 til 6 mánaða aldurs, járnþúskap og hlutfall vatns og fitu í líkama barns við 6 mánaða aldur, vöxt frá fæðingu til 29-38 mánaða aldurs, þroska og hegðun við 18 mánaða og 30-35 mánaða aldur og einnig um heildarlengd brjóstagjafar.

Til þess að geta metið þýðingu brjóstagjafaráðgjafa við val á viðbótarfæði snemma á ungbarnaskeiði og áhrif þess að börn nærast eingöngu á brjóstamjólki fyrstu 6 mánuðina á heildarlengd brjóstagjafar voru einnig notuð gögn úr langtíma ferilrannsókn á börnum fæddum á Íslandi frá janúar til desember 2005 sem valin voru af handahófi af Hagstofu Íslands. Skilyrði fyrir þátttöku í rannsókn þessari voru: einburi, a.m.k. 37 vikna meðganga og að barn væri heilbriggt og án heilbrigðisvanda. Upplýsingum um mataræði var m.a. safnað mánaðarlega frá 5-8 mánaða aldri þar sem foreldrar skráðu fæði barnanna í matardagbók í einn sólarhring. Einnig var upplýsingum safnað um heildarlengd brjóstagjafar.

**Niðurstöður:** Ungbörn í hópi EBF innbyrtu marktækt meira magn af brjóstamjólki við 6 mánaða aldur ( $901 \pm 158$  g/d) samanborið við ungbörnin í hópi CF ( $818 \pm 166$  g/d;  $p=0.012$ ). Niðurstöðurnar breyttust ekki þegar leiðrétt var fyrir þáttum sem voru marktækt frábrugðnir

milli hópa við upphaf rannsóknarinnar. Meðal orkuinntaka fengin úr viðbótarfæði hjá ungbörnum í hópi CF var  $265.4 \pm 219.0$  kJ/d ( $63.4 \pm 52.3$  kcal/d) eða 36.8 kJ/kg líkamspýngdar (8.8 kcal/kg); aðeins 10% af meðal orkuinntöku sem er ráðlögð daglega fyrir 6-11 mánaða gömul ungbörn. Ungbörn í hópi CF voru með hærra forðajárn við 6 mánaða aldur ( $p=0.02$ ) sem var enn marktækt þegar leiðrétt var fyrir þáttum sem voru marktækt frábrugðnir milli hópa við upphaf rannsóknarinnar. Enginn munur var milli hópanna á tíðni járnskortsblóðleysis, járnskorts eða skertum járnbirgðum við 6 mánaða aldur. Enginn munur sást á vextinum milli 4 og 6 mánaða eða líkamssamsetningu við 6 mánaða aldur milli íhlutunarhópanna tveggja.

Eftirfylgni barnanna sýndi að lengd eingöngu brjóstagjafar í 4 samanbórið við 6 mánuði hafði ekki áhrif á þroska eða hegðun við 18 mánaða eða 30-35 mánaða aldur. Samt sem áður, við 30-35 mánaða aldur, höfðu færri foreldrar barna í hópi CF (2%) áhyggjur af grófhreyfingum barna sinna samanbórið við fjölda foreldra barna í hópi EBF (19%;  $p=0.01$ ). Þessi munur var enn marktækur þegar leiðrétt var fyrir þáttum sem voru martækt frábrugðnir milli hópa við upphaf rannsóknar ( $p=0.03$ ). Enn fremur hafði það að gefa viðbótarfæði samhliða brjóstamjólki við 4 mánaða aldur ekki áhrif á tíðni of þungra barna við 18 mánaða ( $p=0.74$ ) eða 29-38 mánaða aldur ( $p=0.36$ ).

Þegar notað var viðbótar þýðið frá langtíma ferilrannsókninni sýndu niðurstöður að það að hafa ótakmarkaðan aðgang að brjóstagjafaráðgjafa leiddi til hægari innleiðingu viðbótarfæðis, sem mælt var með fjölda máltíða á dag við 6 mánaða aldur ( $p=0.001$ ). Ennfremur sást að börn þeirra mæðra sem höfðu ótakmarkaðan aðgang að brjóstagjafaráðgjafa og fengu eingöngu brjóstamjólki í 6 mánuði voru marktækt lengur á brjósti ( $1.08 \pm 0.15$ ) en þau börn sem fengu viðbótarfæði frá 4 mánaða aldri samhliða brjóstamjólki, bæði meðal þeirra sem voru með ótakmarkaðan aðgang að brjóstagjafaráðgjafa ( $1.01 \pm 0.11$ , 95% CI -0.001, 0.16) og þeirra mæðra sem tilheyrðu hefðbundnu ungbarnaefirliti og því ekki með ótakmarkaðan aðgang að brjóstaráðgjafa ( $0.96 \pm 0.13$ , 95% CI 0.03, 0.22). Mæður í báðum rannsóknunum sem gáfu börnum sínum eingöngu brjóstamjólki til 6 mánaða aldurs höfðu svipaða heildarlengd brjóstagjafar, óhað aðgangi þeirra að brjóstagjafaráðgjafa. **Ályktun:** Þar sem sambærileg orkuinntaka sást í báðum íhlutunarhópum þá gefur það til kynna að þau börn sem fá eingöngu brjóstamjólki í 6 mánuði í iðnríkjum séu ekki í aukinni hættu á vaxtarskerðingu á ungbarnaskeiði. Það að bæta við litlu magni af viðbótarfæði samhliða brjóstamjólki frá 4 mánaða aldri hefur ekki áhrif á vaxtarhraða né líkamssamsetningu barnanna við 6 mánaða aldur, en hefur lítil og jákvæð áhrif á járnþéttleik þeirra við 6 mánaða aldur. Brjóstagjafaráðgjafar virðast ýta undir hægari innleiðingu viðbótarfæðis og eingöngu brjóstamjólki í 6 mánuði getur ef til vill aukið heildarlengd brjóstagjafar.

Engin áhrif sást af eingöngu brjóstamjólki í 6 mánuði á þroska eða hegðun við 18 mánaða aldur, þó að við 30-35 mánaða aldur höfðu hlutfallslega færri foreldrar barna sem fengu viðbótarfæði við 4 mánaða aldur áhyggjur af grófhreyfingum barna sinna. Ennfremur sást að eingöngu brjóstamjólki í 4 eða 6 mánuði hafði ekki áhrif á tíðni of þungra barna við 29-38 mánaða aldur. Niðurstöður þessarar rannsóknar undirstrika mikilvægi þess að framkvæma stærri íhlutandi slembiráðaða rannsókn á lengd eingöngu brjóstagjafar.

**Lykilorð:** Eingöngu brjóstamjólki, viðbótarfæði, íhlutandi slembiröðuð rannsókn, brjóstamjólki, járnþéttleikur, vöxtur, þroski, brjóstagjafaráðgjafi, heildarlengd brjóstagjafar



# Table of Contents

<b>ABSTRACT</b>	<b>5</b>
<b>ÁGRIP</b>	<b>7</b>
<b>LIST OF FIGURES</b>	<b>11</b>
<b>LIST OF TABLES</b>	<b>11</b>
<b>ABBREVIATIONS</b>	<b>12</b>
<b>LIST OF ORIGINAL PAPERS</b>	<b>13</b>
<b>1. INTRODUCTION</b>	<b>15</b>
<b>2. BACKGROUND</b>	<b>16</b>
<b>2.1 DIET IN INFANCY</b>	<b>16</b>
2.1.1 HEALTH BENEFITS OF BREASTFEEDING	16
2.1.2 BREAST MILK COMPOSITION	16
2.1.3 OPTIMAL DURATION OF EXCLUSIVE BREASTFEEDING	17
2.1.4 COMPLEMENTARY FEEDING	17
2.1.5 TOTAL BREASTFEEDING DURATION	18
2.1.6 PREVALENCE OF BREASTFEEDING AND EXCLUSIVE BREASTFEEDING	19
2.1.7 BREASTFEEDING SUPPORT	19
<b>2.2 BREAST MILK INTAKE AND ENERGY REQUIREMENTS IN INFANCY</b>	<b>19</b>
2.2.1 ISOTOPE STUDIES	20
<b>2.3 IRON STATUS IN INFANCY</b>	<b>20</b>
2.3.1 FACTORS INFLUENCING IRON STATUS	20
2.3.2 EFFECTS OF IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA	21
2.3.4 IRON STATUS AND EXCLUSIVE BREASTFEEDING	21
<b>2.4 GROWTH IN INFANCY</b>	<b>22</b>
2.4.1 RAPID INFANCY GROWTH	22
2.4.2 GROWTH IN INFANCY AND EXCLUSIVE BREASTFEEDING	22
2.4.3 THE WHO CHILD GROWTH STANDARDS	22
<b>2.5 DURATION OF EXCLUSIVE BREASTFEEDING - EFFECTS IN EARLY CHILDHOOD</b>	<b>23</b>
2.5.1 DEVELOPMENTAL AND BEHAVIORAL STATUS IN EARLY CHILDHOOD	23
2.5.2 OVERWEIGHT AND OBESITY IN EARLY CHILDHOOD	25
<b>3. AIMS</b>	<b>26</b>
<b>4. METHODS</b>	<b>27</b>
<b>4.1 STUDY DESIGN</b>	<b>27</b>
<b>4.2 STUDY POPULATION</b>	<b>28</b>
4.2.1 RANDOMIZATION	28
<b>4.3 DATA COLLECTION</b>	<b>29</b>
4.3.1 ISOTOPE MEASUREMENTS	29
4.3.2 DIETARY ASSESSMENT	31
4.3.3 BLOOD SAMPLING	31
4.3.4 DEVELOPMENTAL OUTCOMES	31
4.3.5 ANTHROPOMETRIC ASSESSMENT	34
<b>4.4 ADDITIONAL NATIONAL PROSPECTIVE COHORT STUDY</b>	<b>35</b>
4.4.1 DATA COLLECTION	35
	9

<b>4.5</b>	<b>STATISTICAL ANALYSIS</b>	<b>35</b>
<b>4.6</b>	<b>APPROVAL</b>	<b>36</b>
<b>5.</b>	<b>RESULTS AND DISCUSSION</b>	<b>37</b>
<b>5.1</b>	<b>BASELINE CHARACTERISTICS OF PARTICIPANTS</b>	<b>37</b>
<b>5.2</b>	<b>DIET IN INFANCY (PAPER I, II)</b>	<b>39</b>
5.2.1	BREAST MILK INTAKE	39
5.2.2	COMPLEMENTARY FEEDING INTAKE	40
<b>5.3</b>	<b>ANTHROPOMETRIC OUTCOMES IN INFANCY (PAPER I, II)</b>	<b>41</b>
<b>5.4</b>	<b>IRON STATUS AT SIX MONTHS (PAPER II)</b>	<b>43</b>
<b>5.5</b>	<b>EFFECTS OF EXCLUSIVE BREASTFEEDING FOR 4 VS. 6 MONTHS IN EARLY CHILDHOOD (PAPER III, IV)</b>	<b>46</b>
5.5.1	DEVELOPMENTAL AND BEHAVIORAL STATUS	46
5.5.2	ANTHROPOMETRIC OUTCOMES	47
<b>5.6</b>	<b>EFFECTS OF BREASTFEEDING SUPPORT (PAPER V)</b>	<b>51</b>
5.6.1	INCLUDING DATA FROM THE ADDITIONAL NATIONAL PROSPECTIVE COHORT STUDY	51
<b>6.</b>	<b>STRENGTHS AND LIMITATIONS</b>	<b>55</b>
<b>7.</b>	<b>CONCLUSIONS</b>	<b>56</b>
<b>8.</b>	<b>FUTURE PERSPECTIVES</b>	<b>57</b>
<b>9.</b>	<b>ACKNOWLEDGEMENTS</b>	<b>58</b>
<b>10.</b>	<b>REFERENCES</b>	<b>60</b>
	<b>PAPERS I – V</b>	<b>79</b>

## List of Figures

<b>Figure 1:</b>	A schematic diagram of the data collection in the study.....	27
<b>Figure 2:</b>	Isotope measurements of breast milk intake at 5.5-6 months and body composition at 6 months of age.....	30
<b>Figure 3:</b>	Developmental screening tests used in the study: the PEDS questionnaire and the Brigance Screens-II.....	33
<b>Figure 4:</b>	Trial profile.....	38
<b>Figure 5:</b>	Box plot of serum ferritin divided into quartiles.....	45
<b>Figure 6:</b>	Mean height-for-age and mean head circumference-for-age from birth to 29-38 months of age calculated in z scores.....	49
<b>Figure 7:</b>	Mean BMI-for-age and mean weight-for-age from birth to 29-38 months of age calculated in z scores.....	50
<b>Figure 8:</b>	Breastfeeding frequency at every age in the two study populations: mother-infant pairs having unlimited access to lactation consultants and those receiving routine care at the well-baby clinics.....	54

## List of Tables

<b>Table 1:</b>	Baseline characteristics of subjects.....	39
<b>Table 2:</b>	Intake of selected nutrients received at 5.25 months of age as an average intake from weighed dietary registration of complementary foods.....	41
<b>Table 3:</b>	Growth rate in z scores from birth to 6 months and body composition at 6 months.....	42
<b>Table 4:</b>	Iron status indices at 6 months.....	43
<b>Table 5:</b>	Selected measures of developmental and behavioral status for children at 18 months and at 30–35 months of age.....	47
<b>Table 6:</b>	BMI-for-age in z scores and the prevalence of children at risk of overweight ( $>1$ SD), were overweight ( $>2$ SD) or obese ( $>3$ SD) at 18 months and 29-38 months of age.....	51
<b>Table 7:</b>	Number of meals at 5 and 6 months of age among infants receiving complementary foods from the age of 4 months in addition to breast milk, having unlimited access to lactation consultant or received routine care at the well-baby clinics.....	52
<b>Table 8:</b>	Selected food types of solid, semi-solid and soft foods infants consumed at approximately 5 months among those who received complementary foods from the age of 4 months in addition to breast milk having unlimited access to lactation consultant or received routine care at the well-baby clinics.....	53

## Abbreviations

WHO	World Health Organization
CF	Complementary feeding
EBF	Exclusive breastfeeding
ID	Iron deficiency
DHA	Docosahexaenoic acid
ARA	Arachidonic acid
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology and Nutrition
AAP	American Academy of Pediatrics
IDA	Iron deficiency anemia
SGA	Small for gestational age
NCHC	National Center for Health Statistics/World Health Organization
CDC	Center for Disease Control
PEDS	Parent's Evaluations of Developmental Status
BMI	Body mass index
IBCLC	International Board Certified Lactation Consultant
Hb	Hemoglobin
MCV	Mean corpuscular volume
RDW	Red blood cell distribution width
SF	Serum ferritin
TIBC	Total iron-binding capacity
SD	Standard deviation
IQR	Interquartile range

## List of original papers

This dissertation is based on the following papers, which will be referred to in the text by their respective Roman numerals:

- I. Wells JC, Jonsdottir OH, Hibberd PL, Fewtrell MS, Thorsdottir I, Eaton S, Lucas A, Gunnlaugsson G, Kleinman RE. Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: differences in breast-milk intake by stable-isotope probe. **Am J Clin Nutr.** 2012;96(1):73-9.
- II. Jonsdottir OH, Thorsdottir I, Hibberd PL, Fewtrell MS, Wells JC, Palsson GI, Lucas A, Gunnlaugsson G, Kleinman RE. Timing of the introduction of complementary foods in infancy: A randomized controlled trial. **Pediatrics.** 2012;130(6):1038-45.
- III. Jonsdottir OH, Thorsdottir I, Gunnlaugsson G, Fewtrell MS, Hibberd PL, Kleinman RE. Exclusive breastfeeding and developmental and behavioral status in early childhood. Submitted.
- IV. Jonsdottir OH, Kleinman ER, Wells JC, Fewtrell MS, Hibberd PL, Gunnlaugsson G, Thorsdottir I. Exclusive breastfeeding for 4 versus 6 months and growth in early childhood. Submitted.
- V. Jonsdottir OH, Fewtrell MS, Gunnlaugsson G, Kleinman RE, Hibberd PL, Jonsdottir JM, Eiriksdottir I, Rognvaldsdottir AM, Thorsdottir I. Initiation of complementary feeding and duration of total breastfeeding: unlimited access to lactation consultants *versus* routine care at the well-baby clinics. Submitted.

Papers included with the permission of publishing journals.



# 1. Introduction

The aim of this PhD thesis is to enhance our understanding of the effects of diet during the first six months of life on health outcomes in infancy and childhood. The Icelandic health system provides the opportunity to all women for excellent pre- and postnatal care. Consequently the prevalence of exclusive breastfeeding in Iceland during early infancy is high; 50% and 35% of mothers exclusively breastfeed through 4 and 5 months of age, respectively. <sup>(1)</sup> Therefore this population of mother-infant pairs provides an opportunity to investigate the effects of the duration of exclusive breastfeeding on both short - and long-term health outcomes in infancy and early childhood.

It has become evident that nutrition in early life may have beneficial effects on health outcomes later in life. <sup>(2,3)</sup> Breast milk is known to be the optimal source of nutrition in early life and the World Health Organization (WHO) recommends exclusive breastfeeding for 6 months and complementary feeding thereafter until 2 years of age, with continued breastfeeding as long as it suits both mother and child. <sup>(4)</sup> In low resource countries exclusive breastfeeding can decrease mortality and morbidity of infants and young children where available water and complementary foods may be nutritionally inadequate or contaminated. <sup>(5-7)</sup> However the benefits of exclusive breastfeeding are not as obvious for children in the resource rich countries and the scientific basis for the recommendations have been questioned. <sup>(8)</sup> The recommendations are mainly based on observational studies. Few randomized controlled trials are available to guide policy decisions in this regard and the WHO has requested randomized trials in this area. <sup>(9)</sup>

Adequate iron in early life is particularly important for growth and development of infants and children. Breast milk is a poor source of iron and susceptible infants might be at risk of iron deficiency (ID) with or without anemia. <sup>(10)</sup> ID early in life, with or without anemia, may have irreversible consequences on the subsequent developmental status of children. <sup>(11-14)</sup> ID is the most common single micronutrient deficiency worldwide, and is particularly problematic in those low resource countries where infants are often born with suboptimal iron stores. <sup>(13)</sup> ID is thought to affect ~ 42% of children under the age of 5 years in low resource countries and 17% in resource rich countries. <sup>(15)</sup>

This thesis addresses relevant questions regarding diet, nutrition, anthropometric outcomes, iron status, developmental and behavioral status and other health related variables during the first 38 months of life of healthy infants and children. The thesis is based on a randomized controlled trial conducted in Iceland which investigated the effects of exclusive breastfeeding for 4 or 6 months on health outcomes in infancy and early childhood.

## 2. Background

### 2.1 Diet in infancy

#### 2.1.1 *Health benefits of breastfeeding*

Breast milk is the optimal sole source of nutrition for infants during early infancy and breastfeeding confers significant immediate and potentially long-term health benefits for both mothers and their infants. Therefore support for breastfeeding is considered to be an important public health measure.<sup>(16)</sup> The most obvious short-term advantage of breastfeeding to infants is the decreased risk from acute gastrointestinal tract infections, lower respiratory infections and otitis media. Breastfeeding is also associated with a lower risk for infant mortality, and allergic diseases.<sup>(16-19)</sup>

Potential long-term advantages of breastfeeding include protection against overweight and obesity, type 2 diabetes, poorer neurodevelopmental outcomes, hypertension and hyperlipidemia.<sup>(17, 20-23)</sup> Breastfeeding may also have maternal advantages, including longer lactational amenorrhea, more weight loss after birth and a decreased risk of type 2 diabetes mellitus and breast and ovarian cancer.<sup>(18, 24-26)</sup> As a result, breastfeeding is also economically beneficial for both families and society.<sup>(27-29)</sup>

#### 2.1.2 *Breast milk composition*

Infants have proportionally high nutritional requirements for their body weight compared to children and adults.<sup>(30)</sup> Breast milk contains all of the macro and micro nutrients, including fat, carbohydrate, protein, vitamins, minerals and water, that the infant needs during the first months of its life. Breast milk is readily digestible by the infant. Compared with commercial infant formulas, breast milk contains more and varied carbohydrates and less protein. Furthermore, the ratio of whey to casein and the quality of both these proteins in human milk differs from those of cow milk. Breast milk also contains the long chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and arachidonic acid (ARA) which participate in critical cell signaling and structural functions. In addition, breast milk, unlike cow milk, contains many bioactive substances that provide protection against various infections. These factors include immunoglobulins, white blood cells, cytokines, transport proteins, nucleotides and oligosaccharides.<sup>(31)</sup>

Breast milk of well nourished mothers, contains levels of essential vitamins and minerals needed for normal full-term infant growth and development, except for vitamin D, which must be provided from exogenous sources, either through exposure to sunlight or as a supplement. If the mother is malnourished then the breast milk may contain lower levels of some vitamins and minerals.<sup>(31)</sup> The concentration of iron and zinc are low in breast milk, regardless of the mother's nutritional status, but they have high bioavailability and absorption from the infant's intestine. Most full-term infants are born with iron endowments that are



thought to provide the infant with iron adequate for growth and development up to 4-6 months of age. The amount of zinc in breast milk declines rapidly between birth to 6 months of age.<sup>(10)</sup>

### 2.1.3 *Optimal duration of exclusive breastfeeding*

There has been a longstanding debate about the optimal duration of exclusive breastfeeding; whether infants should be exclusively breastfed for 4 or 6 months after birth.<sup>(8)</sup> The current recommendations of the WHO are that infants should be exclusively breastfed for the first 6 months of life<sup>(32)</sup> but until May 2001 the WHO recommended exclusive breastfeeding for 4-6 months of age.<sup>(4)</sup> The recommendations were changed following a report by a WHO Expert Consultation on the Optimal Duration of Exclusive Breastfeeding<sup>(33)</sup> and a systematic review.<sup>(9)</sup> The systematic review was based mostly on scientific evidence from observational studies but also included data from a randomized intervention trial and a large cluster-randomized trial from low resource countries, Honduras<sup>(34, 35)</sup> and Belarus, respectively.<sup>(5)</sup>

The principle reasons for changing the recommendation were to provide optimal nutrition while lowering the risk of infectious disease for young infants in low resource countries where available water may be contaminated and complementary foods may be nutritionally inadequate.<sup>(5-7, 36, 37)</sup> While this is particularly important in low resource countries where rates of gastrointestinal tract infections are high<sup>(38)</sup> the evidence for recommending 6 months of exclusive breastfeeding in resource rich countries is less clear.<sup>(8)</sup>

Another systematic review of the optimal age for the introduction of complementary foods concluded that there was a lack of clear evidence to support the change from the earlier WHO recommendations on exclusive breastfeeding from birth to 4-6 months of age.<sup>(39)</sup> The authors of a recent systematic review concluded that further research is needed to determine the risks of undernutrition including iron deficiency among infants exclusively breastfed for 6 months without iron supplementation.<sup>(37)</sup> Others have raised concerns about the need for some flexibility in the infant feeding recommendations because of variations in infant development and readiness for complementary foods, as well as variations in environmental conditions in low resource vs. resource rich countries.<sup>(8)</sup> Few randomized controlled trials are available to guide policy decisions in this regard, a fact recognized by the WHO in a request for more such studies.<sup>(9)</sup>

### 2.1.4 *Complementary feeding*

After 6 months of age it becomes difficult for infants to meet their energy and nutrient needs from breast milk alone. At 6-8, 9-11 and 12-23 months of age additional calorie needs are 200, 300 and 550 kcal per day, respectively, in addition to breast milk.<sup>(31)</sup> The first two years of life is the peak period for growth faltering, micronutrient deficiencies and infectious illnesses.<sup>(40)</sup> Complementary feeding is defined as the foods introduced while the infant is still breastfeeding and is recommended from 6 months until 2 years or beyond (as long as breastfeeding continues) according to the WHO.<sup>(40)</sup> Complementary foods should be rich in iron since over 90% of the daily iron requirement must be supplied by complementary foods.

<sup>(41)</sup> Infants who do not receive iron rich complementary foods or iron supplementation are at great risk of being iron deficient from 6-12 months of age. <sup>(10)</sup> Furthermore, it has been suggested that early (<4 months) or late ( $\geq 7$  months) introduction of complementary foods that contain gluten should be avoided and gluten should be introduced while the infant is still breastfeeding to decrease the risk of celiac disease. <sup>(41)</sup>

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition concluded that complementary foods should not be introduced before 17 weeks and not later than 26 weeks. <sup>(41)</sup> Most mothers in European countries introduce solid foods before 6 months of age. <sup>(42)</sup> A study in Norway showed that 21% of infants were introduced to complementary foods before 4 months of age and that formula fed infants was given complementary foods earlier compared with those who were breastfed. At 4 months and 6 months of age 47% and 91% of the infants, respectively, were introduced to complementary foods. <sup>(43)</sup> One of the main reasons why mothers introduce complementary foods earlier than 6 months to their infant is because they find their infant appear to be hungry and they worry that breast milk alone is not nutritionally adequate for them. <sup>(44)</sup> The factors most associated with early (<4 months) introduction of complementary foods are maternal smoking, young maternal age, low maternal education, low socioeconomic status, no or short duration of breastfeeding and lack of information or advice from health care providers. <sup>(45)</sup>

Some studies suggest that infant feeding patterns may have effects on feeding patterns in childhood. <sup>(46, 47)</sup> In one study, infants receiving solid foods at 4 months of age were more likely to be given “unhealthier” foods at 1 year of age compared with those not given solid foods at 4 months. <sup>(48)</sup> Early nutrition and feeding practices may also have long-term effects on health outcomes later in life and the risk of developing non-communicable diseases in part mediated through nutritional influences on growth. <sup>(2, 3, 49, 50)</sup> Since complementary foods may influence growth in infancy, the age at which they are introduced might therefore affect future health outcomes. <sup>(51)</sup>

### 2.1.5 *Total breastfeeding duration*

The WHO recommends breastfeeding together with adequate complementary foods until 2 years of age or beyond <sup>(32)</sup> and the American Academy of Pediatrics (AAP) recommends breastfeeding for 1 year or beyond. <sup>(16)</sup> Although breastfeeding has a number of advantages both for mother and infant, breastfeeding duration remains low both in low resource and resource rich countries. <sup>(53)</sup> Many socioeconomic factors affect the total duration of breastfeeding. Older, highly educated mothers of normal weight and having more children, living in urban areas, having less income and exclusively breastfeeding their infants for a longer period of time are thought to be more likely to have a longer duration of total breastfeeding. <sup>(27, 43, 53-64)</sup> Mothers with lower education are more likely to smoke cigarettes during pregnancy <sup>(7)</sup> and a negative association has been found between maternal smoking and duration of total breastfeeding. <sup>(54, 65)</sup> Furthermore other even more modifiable factors that influence total breastfeeding duration are the mother’s intention to breastfeed, her breastfeeding self-efficacy and social supports. <sup>(66)</sup>

### *2.1.6 Prevalence of breastfeeding and exclusive breastfeeding*

The prevalence of breastfeeding in Iceland from 2004-2008 was 92% at 2 months of age, 74% at 6 months and 27% at 12 months. The prevalence of exclusive breastfeeding was lower where 75% of Icelandic infants were exclusively breastfed at 2 month of age, 67% at 3 months, 50% at 4 months, 35% at 5 months and only 8% of infants were exclusively breastfed at 6 months. Therefore relatively few mothers seem to follow the current WHO recommendations about exclusive breastfeeding for 6 months. <sup>(1)</sup>

The incidence and prevalence of breastfeeding differs between countries. Australia and Europe have a higher initiation and duration of breastfeeding compared with the United States and Canada. <sup>(67)</sup> The prevalence of breastfeeding in the Nordic countries has increased during recent decades and is relatively high. The prevalence of exclusive breastfeeding at 4 months of age is highest in Denmark at 50%, 44% in Norway and 15% in Finland. <sup>(68)</sup> In the United Kingdom though 64% of infants are breastfed initially and only 19% are still breastfed at 4 months of age. Also 90% of infants are given complementary foods before the age of 4 months <sup>(69)</sup> and in 2005 less than 1% of mothers in the United Kingdom exclusively breastfed their infants for 6 months. <sup>(70)</sup>

### *2.1.7 Breastfeeding support*

Breastfeeding support is critical to breastfeeding success and may therefore increase initiation, duration and exclusivity of breastfeeding. <sup>(71-76)</sup> Many barriers to breastfeeding can be removed with breastfeeding support through lactation consultation <sup>(63)</sup> leading to longer breastfeeding duration. Prenatal and postnatal counseling and support to breastfeeding mothers is thought to be one of the main factors in extending breastfeeding duration. <sup>(77-82)</sup> A recent systematic review showed that professional support at the individual level increased the rate of breastfeeding from 4 to 5 months, a time when breastfeeding rates typically decline sharply. <sup>(83)</sup> Lactation consultants can maintain breastfeeding among mothers through encouragement and support. <sup>(84)</sup> A recent longitudinal study, conducted in Glasgow, showed that mothers who exclusively breastfed their infants and received breastfeeding support, increased their breast milk volume between 4 and 6 months. <sup>(85)</sup>

## **2.2 Breast milk intake and energy requirements in infancy**

There has been a longstanding debate over the optimal duration of exclusive breastfeeding with some expressing concerns about the nutritional adequacy of breastmilk as the sole source of nutrition for the infant for the entire first six months of life. <sup>(8, 37)</sup> Suitable infant energy balance is important for infant development. Estimated average daily energy requirements are 390, 365 and 355 kJ/kg body weight at 1, 3 and 6-11 months, respectively. <sup>(68)</sup> According to the WHO, the daily energy requirement for breastfed infants at 6 months is

78 kcal/kg body weight (326 kJ/kg)<sup>(86)</sup> and the energy in breast milk is assumed to be 0.67 kcal/g at 6 months (2.8 kJ/kg).<sup>(10)</sup> The WHO recommendation for breast milk intake for exclusively breastfed infants at 6 months is 854 g/d. These values are derived from 8 observational studies in resource rich countries and are based on 24-hour test-weighing of breast milk from highly selected populations<sup>(10)</sup> and therefore raise concerns about the generalizability of these reference values for the general population. According to published data the mean metabolisable energy content of breast milk is 2.6 kJ/g of breast milk and at 5 and 6 months the weighted mean of breast milk intake among infants was 827 and 894 g/d, respectively. Breast milk was shown to supply on average about 90% of infants energy requirements at 6 months of age among those who were exclusively breastfed and therefore energy from breast milk might be inadequate to meet some infant's energy requirements at 6 months.<sup>(87)</sup> However, there are a lack of data regarding the energy content of breast milk and the amount of breast milk exclusively breastfed infants consume at 6 months of age.

### 2.2.1 *Isotope studies*

To estimate total energy intakes among breastfeeding infants it is important to know the intakes and energy content of breast milk. Sufficient energy must be consumed in infancy to support rapid growth as well in addition to homeostasis and activity. However, it is difficult to accurately determine nutrient intakes in exclusively breastfed infants, if the intake of breast milk is not measured directly.<sup>(10)</sup> Unfortunately, the test weighing method of direct measurement is invasive for both mothers and infants and can bias the results of breast milk intake.<sup>(88)</sup> Objective data on breast milk intakes are lacking.<sup>(10, 89)</sup> Non-invasive, stable isotope methods have been used to measure breast milk intake over a 14 day period since the report by Coward et al. in 1979.<sup>(90)</sup> Measuring breast milk intake with stable isotope techniques has provided direct reproducible data on breast milk intake in various populations around the world.<sup>(89)</sup> The stable isotope techniques involves using a nonradioactive tracer, deuterium labeled water ( $^2\text{H}_2\text{O}$ ), to determine the rate of the flux of water from mother to infant and of water efflux. The dose of isotopes is administered orally to the mother and urine samples are obtained from both mother and infant over the following 2 weeks to measure breast milk intake.<sup>(89-91)</sup>

## 2.3 **Iron status in infancy**

### 2.3.1 *Factors influencing iron status*

Many factors can influence the iron status of infants and children. ID is most prevalent during times of rapid growth in infancy, adolescence, during pregnancy and in women during childbearing years.<sup>(68)</sup> Rapid growth during the period from birth to two years of age causes a greater need for iron. Studies have shown that infants with higher growth rates are at greater risk of ID<sup>(92-94)</sup> thought to be accounted for by more rapidly depleted iron stores from birth with greater weight gain.<sup>(95)</sup> A positive relationship has been seen between iron status at 6

months and birth weight<sup>(93, 96)</sup> but this relationship was not seen in 1 year old Icelandic infants.<sup>(97)</sup> It is well known that birth weight may influence iron endowment at birth.<sup>(98)</sup> Low birth weight infants often have low iron stores at birth and furthermore they tend to grow faster which can increase the risk of developing ID and iron deficiency anemia (IDA). Infants who are born weighing between 2000-2500 g represent 3-5% of all infants in affluent countries.<sup>(99)</sup> It has been previously reported that delayed umbilical cord clamping after birth can have a beneficial effect on the infant's iron status,<sup>(100)</sup> even up to 6 months of age.<sup>(101, 102)</sup>

Gender is also associated with iron status where boys tend to have worse iron status than girls in early infancy and they seem to be more prone to be iron deficient.<sup>(94, 97, 103, 104)</sup> This gender difference has even been seen among infants beyond 6 months of age.<sup>(105)</sup> However there are multiple factors that influence the risk of ID in infancy, including premature birth, poverty,<sup>(93)</sup> early cord clamping after birth and poor iron status before pregnancy.<sup>(104, 106)</sup> If all these factors are mitigated than adequate iron status may be maintained until 6 months of age,<sup>(98)</sup> but if not then ID and IDA may be ongoing for months before the infant receives iron from dietary sources.<sup>(107)</sup>

### 2.3.2 *Effects of iron deficiency and iron deficiency anemia*

It is well documented that breastfed infants can develop ID and IDA before 6 months of age,<sup>(107-113)</sup> even those who are full term and weigh >2500 g at birth.<sup>(99, 107)</sup> IDA in early life can have both short – and long term effects on the development of the brain that may not be reversible, although this remains controversial.<sup>(11-14)</sup> Children who suffer from IDA in infancy can have poorer cognitive skills and impaired motor development, even in childhood.<sup>(114, 115)</sup> Furthermore, infants with ID without anemia may exhibit poorer motor skills.<sup>(116)</sup> Thus it is of great importance to ensure that infants receive sufficient iron in early infancy. Iron supplementation and iron fortified complementary foods are thought to be efficient in the prevention of ID and IDA.<sup>(117)</sup>

### 2.3.4 *Iron status and exclusive breastfeeding*

Iron endowment at birth is thought to be adequate for growth and development until 4-6 months postpartum. The iron obtained from breast milk is highly bioavailable,<sup>(118, 119)</sup> however breast milk is low in iron and declines rapidly from the age of 4- 6 months. Therefore the infant has to receive iron from exogenous sources when the iron stores from birth are exhausted.<sup>(10)</sup> At the same time the new WHO recommendation were put forward it was recognized that susceptible infants with any of the risk factors mentioned above, might be at increased risk of iron deficiency when exclusively breastfed for 6 months.<sup>(9)</sup> Authors of a recent systematic review concluded that infants exclusively breastfed for 6 months living in low resource countries, where their iron stores at birth may be suboptimal, are at greater risk of worse hematologic status if other sources of iron are not provided.<sup>(37)</sup> A randomized intervention trial conducted in a low resource country setting, Honduras, showed that exclusively breastfed infants had poorer iron status at 6 months than those who received

complementary foods from the age of 4 months.<sup>(93)</sup> Other studies have also shown that infants who are exclusively breastfed for 6 months may be at risk of developing ID.<sup>(120, 121)</sup>

## **2.4 Growth in infancy**

### *2.4.1 Rapid infancy growth*

Intrauterine growth restriction may occur because of a low supply of nutrition or oxygen, resulting in low birth weight.<sup>(122)</sup> Rapid postnatal growth is often seen among low birth weight infants and those born small for gestational age (SGA). However, rapid growth in infancy has also been seen among infants who have an appropriate birth weight for their gestational age.<sup>(123, 124)</sup> Studies have demonstrated that rapid growth in infancy might be related to an increased risk for chronic adult diseases.<sup>(125-127)</sup> Authors of a recent systematic review concluded that factors associated with rapid growth in infancy include maternal primiparity, maternal smoking during pregnancy, low birth weight and early introduction of complementary foods.<sup>(128)</sup> Studies suggest that rapid weight gain in formula fed infants may be due to their higher protein intake, although both breastfed and formula fed infants gain weight at the same rate for the first 4 months of life.<sup>(129-133)</sup> Thus one speculation is that longer duration of total and exclusive breastfeeding, and therefore lower protein intake, may result in slower weight gain in later infancy and be protective against later overweight and obesity.<sup>(26, 133-136)</sup>

### *2.4.2 Growth in infancy and exclusive breastfeeding*

Results from a recent systematic review indicated that in both low resource and resource rich countries no deficits were seen in growth among infants exclusively breastfed for the first 6 months of life.<sup>(37)</sup> Furthermore a randomized controlled trial, conducted in a developing country, showed no difference in weight gain up to 12 months of age between infants exclusively breastfed for 4 months compared with those exclusively breastfed for 6 months.<sup>(35)</sup> An observational study conducted in Sweden showed no difference in growth the first half year among infants exclusively breastfed for 6 months compared with those not exclusively breastfed.<sup>(137)</sup> Some studies though indicate that longer duration of exclusive breastfeeding is negatively related to infant weight gain,<sup>(46, 69, 128, 138-140)</sup> In a very recent systematic review, it was concluded that exclusive breastfeeding for longer than 4 months may be associated with slower weight gain in late infancy.<sup>(141)</sup> However, existing evidence on breastfeeding and weight gain in infancy are based on observational studies and may be biased by confounding variables.<sup>(37, 142-144)</sup>

### *2.4.3 The WHO child growth standards*

In 2006 the WHO recommended the universal use of its new growth standards. Previously, the 1977 National Center for Health Statistics/World Health Organization (NCHS/WHO)

growth reference had been recommended for use as an international growth reference, until the year 2000 and 2006 when the U.S. Center for Disease Control (CDC) and the WHO, respectively, released new growth references. The newest WHO standards describe the growth of healthy infants under optimal conditions in contrast to the CDC references that describe growth in the general population of infants and children.<sup>(145)</sup> New global WHO Child Growth Standards for children aged 0-59 months were developed using data from the WHO Multicentre Growth Reference Study. The study was conducted between 1997 and 2003 in 6 countries in different geographical regions (Brazil, Ghana, India, Norway, Oman and the United States). The design of the study combined longitudinal follow-up of 882 infants from birth to 24 months and a cross-sectional study design of 6669 children aged 18-71 months. The WHO Child Growth Standards were then developed on the basis of this multi-ethnic sample. Weight-for-age, length/height-for-age, weight-for-length/height and BMI-for-age percentile and z score values were generated for boys and girls.<sup>(146)</sup> Because the former growth reference, NCHS/WHO, was based on predominantly formula fed infants, breastfed infants and children may not be appropriately classified based on that reference population.<sup>(147)</sup> For that reason, the new WHO growth standards were introduced to have a growth reference based on healthy breastfed infants. The WHO Child Growth Standards describe the optimal growth of a healthy breastfed infant living in a hygienic environment, irrespective of ethnicity or geography.<sup>(148)</sup> Furthermore, the WHO Child Growth Standards show that all infants and young children worldwide have the potential to grow at a similar rate in a safe hygienic environment.<sup>(149)</sup> The WHO Child Growth Standards are an important tool for prevention and early detection of overweight and wasting.

## **2.5 Duration of exclusive breastfeeding - effects in early childhood**

### **2.5.1 *Developmental and behavioral status in early childhood***

Studies indicate that breastfeeding may have a positive effect on later cognitive development<sup>(20)</sup> and that the positive effect of breastfeeding may be dose-responsive.<sup>(17)</sup> However this has not been a consistent finding<sup>(18)</sup> and evidence from higher quality studies is less convincing.<sup>(23)</sup> Furthermore, some studies indicate that a longer duration of exclusive breastfeeding is important for this positive association with developmental outcomes in childhood, especially for those born SGA.<sup>(150-152)</sup> Less is known about the impact of breastfeeding and the duration of exclusive breastfeeding on non-cognitive developmental and behavioral status in childhood. Studies indicate that breastfeeding in general and also longer duration of breastfeeding may be associated with a decreased risk of behavioral problems and developmental delays in childhood.<sup>(153-155)</sup> However findings on this subject are inconsistent.<sup>(156)</sup> A large breastfeeding promotion intervention in Belarus showed no relationship between prolonged breastfeeding or longer duration of exclusive breastfeeding and childrens' behavior at 6.5 years of age.<sup>(157)</sup> Other studies have shown that increased duration and exclusivity of breastfeeding may have beneficial effects on language and motor development in childhood.<sup>(55, 158-164)</sup>

Developmental status is influenced by a number of genetic and environmental factors that cause cumulative risk effects for developmental delays that are generally not addressed in observational studies. This is one possible explanation for the inconsistent findings among such studies, with some that show no relationship between breastfeeding and development while others show an association.<sup>(158, 165, 166)</sup> Studies investigating the relationship between breastfeeding and developmental status often compare formula fed infants to breastfed infants, both those born at term<sup>(167, 168)</sup> and those born prematurely or SGA,<sup>(166, 169)</sup> but less is known of the impact of exclusive breastfeeding compared to partial breastfeeding.

#### *2.5.1.1 Screening tools for developmental-behavioral status of children*

Screening tools are used to evaluate the developmental and behavioral status, among other things, of a large number of children to identify which children may be in need for further evaluation. Interventions for developmental delay though should not be based solely on results of screening tests. Early detection of developmental delay and appropriate intervention has been shown to be effective in improving developmental outcomes in childhood.<sup>(170)</sup> In 2006, the AAP recommended systematic developmental screening in the primary care setting using a validated screening tool for children aged 9, 18 and 30 months, but no recommendations were provided for the specific screening tools that should be used.<sup>(171)</sup>

##### *2.5.1.1.1 Brigance early preschool Screen-II (The Brigance Screens-II)*

The Brigance Screens-II is a comprehensive, reliable and valid screening tool of developmental status that is completed by the child itself.<sup>(171, 172)</sup> General developmental screening tools similar to the Brigance Screens-II that are directly administered to the child and are suitable for primary care settings are the Battelle Developmental Inventory Screening Tool Test II, the Bayley Infant Neurodevelopmental Screener and the Denver-II Developmental Screening Test, which are all comparable to the Brigance Screens-II in sensitivity and specificity.<sup>(171, 173)</sup> All of these screening instruments measure multiple domains of behavioral, motor and cognitive development.

##### *2.5.1.1.2 Parent's Evaluation of Developmental Status (PEDS)*

The PEDS questionnaire is answered by the parents and is therefore solely based on parental perception of their children's developmental and behavioral status. A positive correlation has been shown between the results of the PEDS questionnaire and the Brigance Screens-II.<sup>(174)</sup> The PEDS questionnaire is a valid and reliable developmental screening tool<sup>(171)</sup> and the value of parents' concerns in the detection of developmental delay has been well studied.<sup>(175, 176)</sup> Less educated parents and those with psychosocial risk factors are less likely to address their concerns about their child's development to professionals.<sup>(175)</sup> Commonly used parent-completed screening questionnaires in primary care settings comparable to the PEDS questionnaire are the Ages & Stages Questionnaires, the Child Development Review-Parent Questionnaire and the Infant Development Inventory.<sup>(171, 173)</sup> These tests are not perfectly



concordant, but are widely used and are considered appropriate for developmental evaluation of children in primary care settings. <sup>(177, 178)</sup>

### 2.5.2 *Overweight and obesity in early childhood*

Obesity in childhood is a significant problem globally. It has been shown that children who are overweight are more likely to be obese in adulthood and are therefore at greater risk of associated disorders including type 2 diabetes, joint diseases, cancer and cardiovascular disease. Rapid weight gain in infancy is thought to be a risk factor for overweight and obesity in childhood and later in life in resource rich countries. <sup>(131, 179-183)</sup> Studies investigating the relationship between nutrition in infancy and the later risk of being overweight or obese most often compare breastfeeding and formula feeding. Less is though known about the effect of duration of exclusive breastfeeding on the risk of overweight and obesity in childhood. Some studies indicate that increased duration of exclusive breastfeeding may prevent rapid weight gain in infancy and be protective against overweight and obesity later in life. <sup>(69, 138, 141, 184-186)</sup> A cross-sectional study in eight European countries showed that exclusive breastfeeding for 6 months was thought to be more protective of childhood overweight and obesity compared with exclusive breastfeeding for 4 and 5 months. <sup>(187)</sup> However, studies are inconsistent about whether duration of exclusive breastfeeding affects the risk of being overweight or obese in childhood, and some studies show no relationship between these outcomes and exclusive breastfeeding. <sup>(20, 35, 186, 188-193)</sup> A large cluster-randomized trial of a breastfeeding promotion in Belarus showed no difference in mean body mass index (BMI) and the prevalence of obesity at 6.5 years of age with increased total duration and exclusivity of breastfeeding. <sup>(194)</sup> The protective effects of breastfeeding on overweight might partially be explained by differences in the way mothers oversee their children's diets with more restrictive feeding practices being associated with a higher risk of obesity. <sup>(195, 196)</sup>

### **3. Aims**

The principle aim of this thesis is to enhance our understanding of the effects of diet during the first six months of life on health outcomes in infancy and early childhood.

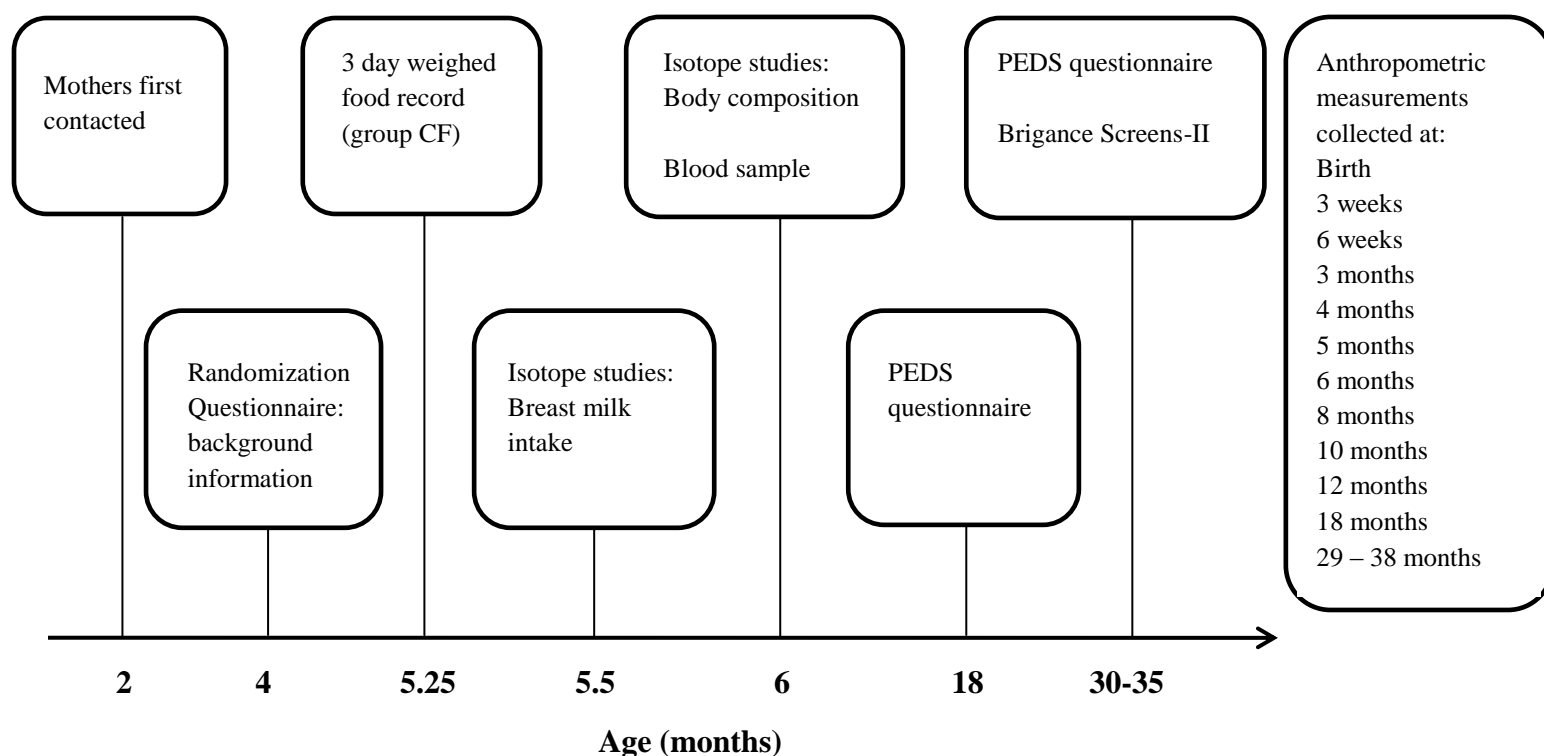
*The specific aims are:*

- ✓ To investigate the effects of the duration of exclusive breastfeeding for 4 vs. 6 months on breast milk intake at 5.5-6 months of age and infant's body composition at 6 months (Paper I).
- ✓ To investigate the effects of the duration of exclusive breastfeeding for 4 vs. 6 months on iron status at 6 months of age and growth from birth to 6 months (Paper II).
- ✓ To investigate the long-term effects of duration of exclusive breastfeeding for 4 vs. 6 months on selected measures of developmental and behavioral outcomes at both 18 and 30–35 months of age (Paper III).
- ✓ To investigate the long-term effect of duration of exclusive breastfeeding for 4 vs. 6 months on growth up to 38 months of age (Paper IV).
- ✓ To investigate the effects of breastfeeding support by a lactational consultant on initial complementary feeding and total breastfeeding duration (Paper V).

## 4. Methods

### 4.1 Study design

Subjects were 119 mother-infant pairs in this parallel group, masked, randomized controlled trial which was conducted in 7 healthcare centers in Iceland. Inclusion criteria in the study for infants were singleton birth, gestational length  $\geq 37$  weeks, exclusively breastfed at the time of screening, infant characterized as healthy based on absence of congenital abnormalities or chronic health issues likely to affect growth, development or iron status. Furthermore, mothers had to be willing to be randomized either to continue exclusive breast feeding for 6 months or to introduce complementary food at the age of 4 months. Background information of the mother, including maternal age and education, parity and mode of delivery, were collected on eligible mother-infant pairs (**Figure 1**).



**Figure 1.** A schematic diagram of the data collection in the study.

## 4.2 Study population

Mothers of eligible infants were informed about the study when their infant was approximately 2 months old and given study handouts if the infant was still exclusively breastfed at 3 months of age. They were contacted before the infant's 4 month birthday and if they were still exclusively breastfeeding, they were invited to participate in the trial. Mothers and their infants attended a screening visit and after obtaining informed consent were evaluated for the following additional eligibility criteria: exclusively breastfed at the time of screening, mother willing to continue to breastfeed exclusively until 6 months or give complementary foods at 4 months based on randomization and mother-infant pair likely able to participate for the duration of the study. Exclusive breastfeeding was defined as breastfeeding with no additional liquid or solid foods other than vitamins and medications.

<sup>(197)</sup> To operationalize this definition in the Icelandic population, the use of up to 10 feedings of formula or water during the first 6 months was allowed to avoid having to exclude infants that in fact were otherwise exclusively breastfed. Eligible mother-infant pairs were randomly assigned to receive complementary foods from the age of 4 months in addition to breast milk (CF), or to continue being exclusively breastfed to the age of 6 months (EBF). Vitamin D supplements were recommended in both groups. Mothers in both groups received counseling from a nurse who also was an International Board Certified Lactation Consultant (IBCLC). Mothers in the EBF group were advised to exclusively breastfeed for 6 months and mothers in the CF group were advised to give the infant complementary foods within 7 days of randomization, alongside continued breastfeeding. During the 2 month study period, mothers could at all times contact the research staff at the Unit for Nutrition Research and the nurse at the healthcare centers.

### 4.2.1 Randomization

The trial statistician provided a computer generated randomization code. Assignments were generated using permuted blocks of two and four, with the sequence presented in random order. Assignments were accessed using a password-protected web-based application, after eligibility criteria were confirmed. The statistician generating these assignments was not involved in any other aspect of the study. Following randomization, one mother-infant pair was randomized to the CF group but was incorrectly instructed to exclusively breastfeed for 6 months, which she did, so in the main analyses this infant was analyzed in the EBF group. The nurses who performed the randomization at the health care centers were not blinded to the participant group status. All isotopic modeling and mass spectrometric analysis were however blinded.

### 4.3 Data collection

The primary outcomes in the study were mean daily breast milk consumption over a two week period, from 5.5 to 6 months. Secondary outcomes included infant body composition, growth and iron status at 6 months. In addition, these children continue to be followed in a longitudinal cohort for nutritional intakes, permitting study of developmental and behavioral outcomes at both 18 and 30–35 months of age, anthropometric outcomes from birth to 29-38 months of age and total breastfeeding duration.

#### 4.3.1 *Isotope measurements*

##### 4.3.1.1 *Breast milk intake*

Dose of the deuterium oxide for mother and infant was weighed on a scale with 0.01g accuracy (Mettler Toledo EL202/00). Research staff from the Unit for Nutrition Research did prepare the dose of the deuterium oxide for all mothers and infants. For all doses prepared a pre dose sample was taken and stored at -20 °C at the Landspítali University Hospital of Iceland. Dose to the mother deuterium oxide turnover method was used to determine breast milk intake.<sup>(91, 198, 199)</sup> This methods also allows estimation of non-breast milk water intake. At the infants age of 5.5 months each mother received orally accurately weighed 10 g deuterium oxide ( $^2\text{H}^2\text{O}$ ) diluted in 50 g of ordinary drinking water, which enabled us to calculate the amount of breast milk intake over a 2 week period, until the infant was 6 months old. The amount of isotope dose the mother consumed was determined by weighing the bottle accurate to 0.01g before and after dosing.

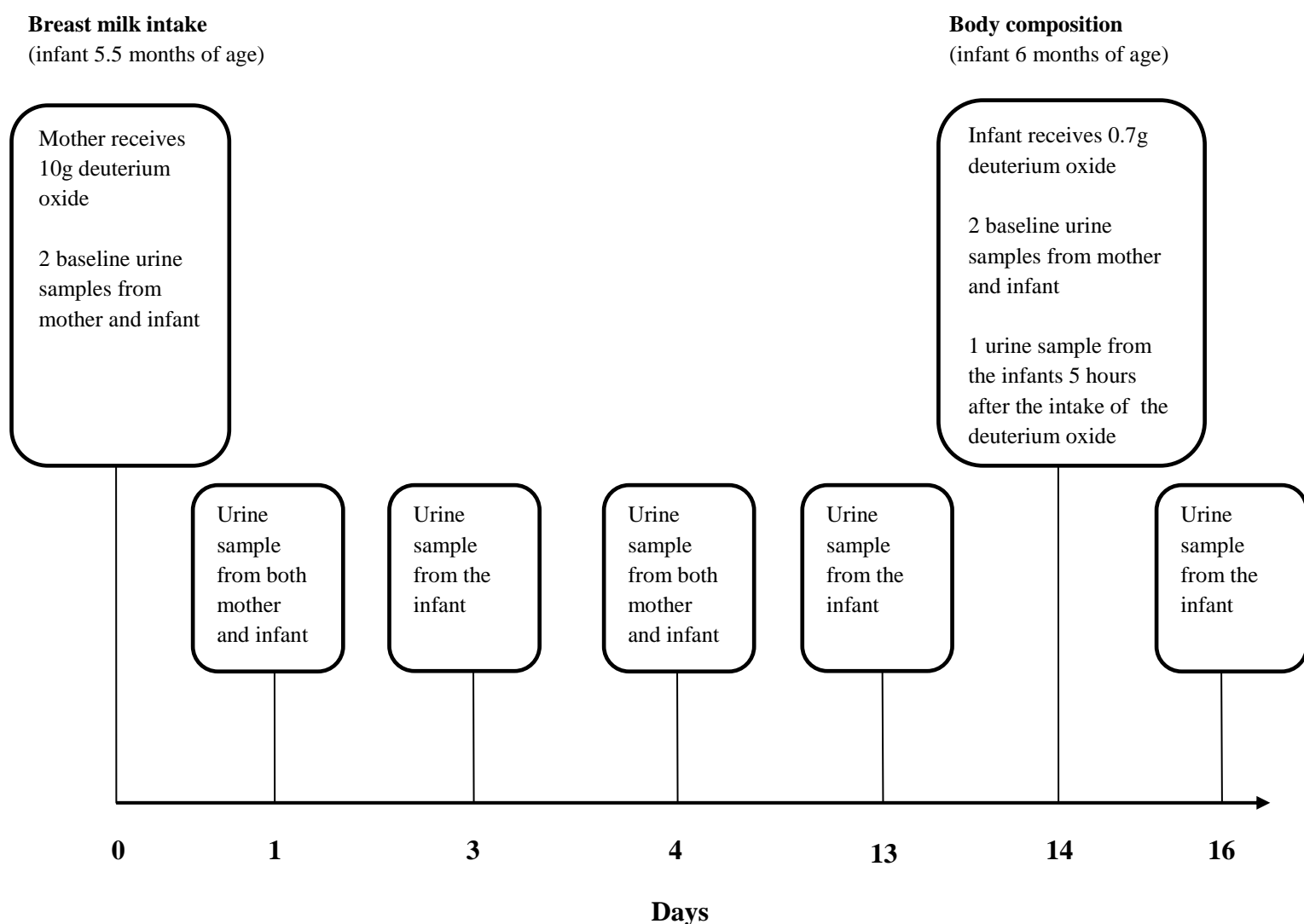
Research staff from the Unit for Nutrition Research showed the mothers how to collect the urine samples and the mothers collected all urine samples, both from themselves and their infants. Urine samples from the infant were obtained by placing 4-5 cotton wool balls in the clean nappy, which the mother checked every half hour and determined if they were wet, so that it would be possible to obtain a timing of the urine samples with maximum 15 minutes margin of error. The timing recorded of the infant's urine samples was the average of the time when the cotton ball was dry until it was checked and noted to be wet. After urination the wet cotton wool balls were retrieved and put into a syringe and the urine then transferred to a 2 ml collection tube. The mother was asked to collect her urine in a small glass that was then put into a collection tube. The mother collected 2 baseline urine samples from the infant and herself before the mother received the dose of deuterium oxide and also on days 1, 3, 4, 13 and 14 from the infant and on days 1, 4 and 14 from herself from the intake of the water containing 10 g deuterium oxide. Taking urine samples was completely painless for both mother and child (**Figure 2**).

The urine samples were kept frozen at the mothers home until all samples had been collected. Then all urine samples were brought to the Landspítali University Hospital of Iceland and stored there at -20 °C until all urine and pre dose samples were transported frozen to the United Kingdom to be analyzed by isotope ratio mass spectrometry (Delta XP,

Thermofisher Scientific, Bremen, Germany). The urine samples were then examined in regard of the amount of deuterium oxide in the urine.

#### 4.3.1.2 *Body composition*

To estimate body fat and lean mass in the infants bodies at 6 months of age, the infants received orally about 0.7 g of deuterium oxide ( $^2\text{H}^2\text{O}$ ; 0.05 g per kg body weight) at the age of 6 months (on day 14 after the mothers intake of the isotope dose). The amount of isotope dose the infant consumed was determined by weighing the bottle and tissue accurate to 0.01 g before and after dosing. The mother collected urine samples from the infant, as previously described, on days 14 (5 hours after the intake of the isotope dose) and 16 after the mothers intake of the isotope dose.



**Figure 2.** Isotope measurements of breast milk intake at 5.5-6 months and body composition at 6 months of age.

#### 4.3.1.3 *Modelling*

Total body water was calculated using the back extrapolation method in both mother and infant.<sup>(200)</sup> It was assumed that the deuterium dilution spaces overestimated total body water by a factor of 1.044.<sup>(201)</sup> Breast milk intake was calculated by fitting the isotopic (tracer) data to a model for water (trace) turnover in the mothers and infants, and the transfer of breast milk from mother to infant, based on equations and assumptions.<sup>(91, 198)</sup> A ‘solver’ function in Excel<sup>TM</sup> was used to minimize the sum of squares of differences between observed and fitted values for maternal and infant data combined.

#### 4.3.2 *Dietary assessment*

Mothers assigned to the CF group were asked to keep a diary to indicate the date that every new food item and new meals were added to the infant’s diet from the time of enrollment into the study until the child was 6 months of age. A 3 day weighed food record was obtained when the infant reached approximately 5 months and 1 week of age ( $\pm 7$  days). Parents were asked to weigh and record every food their infant consumed over this 3 day period, using electronic scales with 1g accuracy (Philips HR 2385, Philips Corporation, Hungary) provided by the study team. Energy and nutrient information were calculated with the nutrient calculation software ICEFOOD (version 2002; Public Health Institute of Iceland, Reykjavik, Iceland), with added information about infant foods.<sup>(202)</sup>

#### 4.3.3 *Blood sampling*

Blood samples were obtained for analyzing iron status. Blood for hemoglobin (Hb), mean corpuscular volume (MCV), red blood cell distribution width (RDW), serum ferritin (SF) and total iron-binding capacity (TIBC) was obtained from the infants at the age of 6 months ( $\pm 7$  days). Analyses were done using a Coulter Counter STKS at Landspítali National University Hospital of Iceland. The cut-off for IDA required that all 3 indicators met the following cut-points: Hb<105 g/l, MCV<74 fl and SF<12  $\mu$ g/l. The same cut-points were used to evaluate both ID: MCV<74 fl and SF<12  $\mu$ g/l; and iron depletion: SF<12  $\mu$ g/l. The iron status indicators and reference values chosen in the study are in line with studies on infant populations in the Nordic countries and previous Icelandic infant studies.<sup>(68, 97, 113, 203)</sup> The blood samples were taken by experienced pediatrician and the infants were only stuck once.

#### 4.3.4 *Developmental outcomes*

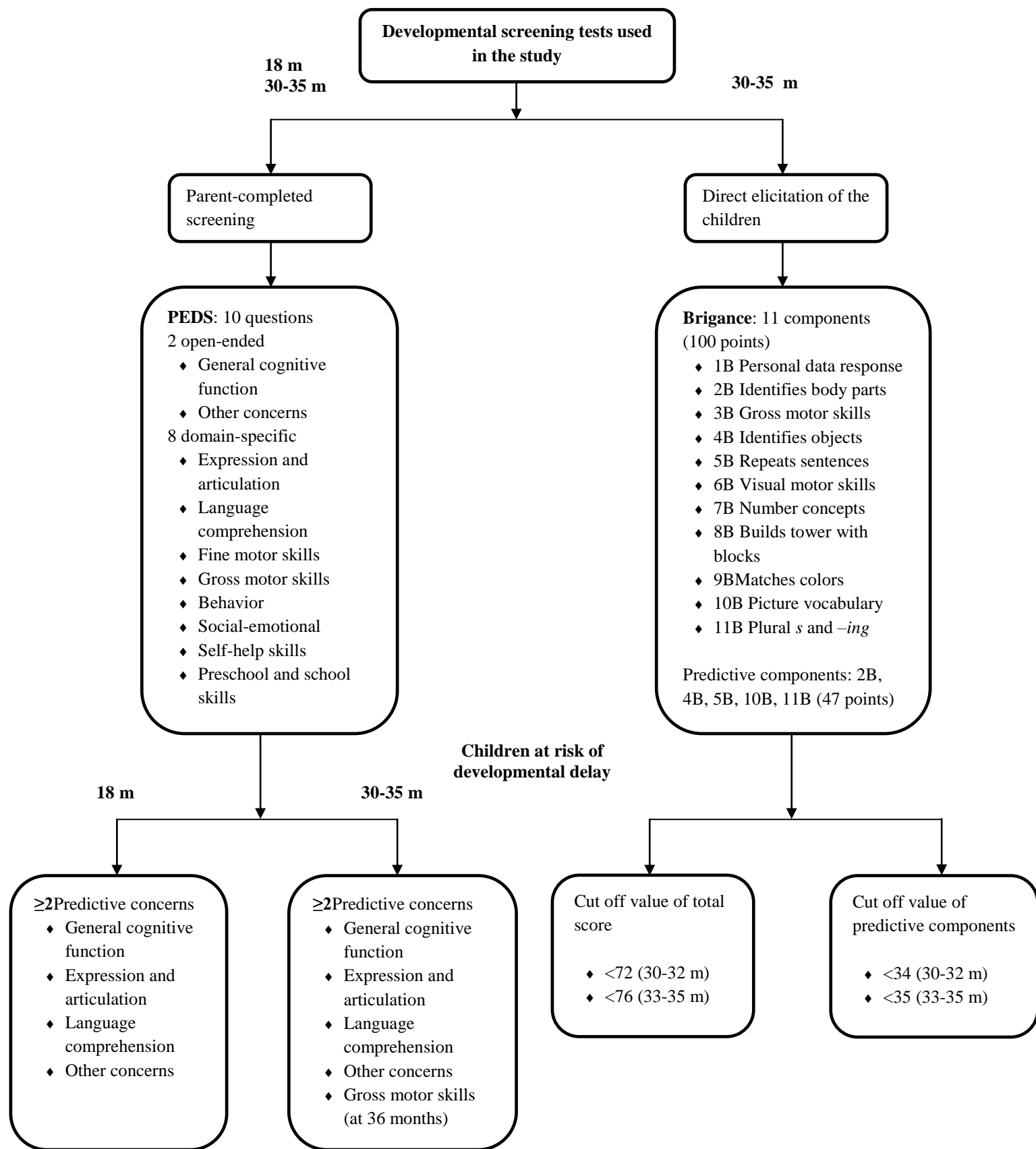
During the children’s routine health care visits at the health care center at 18 months and 30–35 months of age, cognitive development was assessed with both the Parent’s Evaluation of Developmental Status (PEDS) questionnaire and the Brigance early childhood screening test (Brigance Screens-II). The parents filled out the PEDS questionnaire at both visits, at 18 months and 30–35 months of age, and the children underwent the Brigance Screens-II at 30–35 months of age. Both the PEDS questionnaire and Brigance Screens-II were administered by trained nurses at each health care center following prescribed protocols (**Figure 3**). PEDS

questionnaire and Brigance Screens-II were both introduced in 2010 as a part of routine health care visits at health centers in Iceland.

#### *4.3.4.1 Parent's Evaluation of Developmental Status (PEDS)*

The PEDS questionnaire is an evidence-based tool that is designed to detect parental concerns about the developmental status and behaviors of their child. The parent-completed screening questionnaire has been found to have very good reliability and has been validated for children from birth to 8 years of age.<sup>(204-207)</sup> The PEDS questionnaire consists of 10 brief questions, 2 open-ended questions about general cognitive function and other concerns and 8 domain specific items. For each of the 8 domain-specific questions the parents are asked if they have any concerns about the development or behavior of their child and their response option is in a multiple-choice format (no, yes, a little). These questions elicit parental concerns about various aspects of their child's developmental and behavioral status including: expression and articulation, language comprehension, behavior, social-emotional development, self-help skills, preschool and school skills and fine and gross motor skills. Parents are also asked to make general comments about each question. In the beginning and the end of the questionnaire parents are also encouraged to address other concerns related to their children's developmental and behavioral status. Certain parental expressions of concern in response to certain of these questions are more associated with an increased risk of developmental problems and are therefore predictive of developmental delay.<sup>(208)</sup> If parents express concern in response to  $\geq 2$  of these predictive questions (general cognitive function, expression and articulation, language comprehension or express other concerns when the child is 18 months of age and/or have concerns about gross motor skills at 3 years of age) then health center procedures require that the child be referred for further evaluation. It takes parents approximately 5 minutes to answer the questionnaire.





**Figure 3.** Developmental screening tests used in the study: the PEDS questionnaire and the Brigance Screens-II.

#### 4.3.4.2 *Brigance screening test*

The Brigance Screens-II is administered by a trained nurse who observes the child and questions his/her parents and the test is completed by the child itself. It has good reliability and has been validated for measuring the developmental and behavioral status of toddlers and preschool children.<sup>(172, 209, 210)</sup> The Brigance Screens-II for 30-35 months old children is called Brigance, Early Preschool Screen II and is for children from the age of 29 months plus 15 days to 35 months plus 14 days old children. The purpose of the Brigance Screens-II is to identify children with developmental delay who should then be referred for a more comprehensive evaluation. The Brigance Screens-II comprises 11 components that evaluate preschool and school skills, communication skills and motor skills. It takes children approximately 15-20 minutes to complete the test. The highest possible score from the Brigance Screens-II is 100 points. The cut off points for defining children at risk of developmental delay are <72 and <76 points out of 100 for children aged 30-32 months and 33-35 months, respectively. As with the PEDS questionnaire, there are some components of the Brigance Screens-II that are more predictive for developmental delay than others. These include: identifies body parts (2B), identifies objects (4B), repeats sentences (5B), picture vocabulary (10B), plural *s* and *-ing* (11B). The maximum score obtainable from these components of the test is 47 points, combined. Cut off points for defining children at risk of developmental delay are <34 points for children aged 30-32 months of age and <35 points for children aged 33-35 months of age.<sup>(211)</sup> In the current study we focused on assessment of gross motor skills (3B), fine motor skills (6B, 8B) and expressive language (2B, 4B, 5B, 10B, 11B). Both these developmental screening tests have not yet been validated in Iceland.

#### 4.3.5 *Anthropometric assessment*

Infants' weight, length and head circumference were measured at birth, 6 weeks, 3, 4, 5 and 6 months of age at their healthcare centre. Mothers who enrolled in the study were asked if these records could be accessed to determine their infant's growth rate for the trial period. Furthermore, at 5 months postpartum the mothers were weighed at the health care center by the nurse or provided a self-reported weight. After the intervention trial was finished, the participants formed a cohort and additional data was obtained periodically from records of routine care visits at their health care centers after the completion of trial. Additional data on infant's weight, length and head circumference at 8, 10, 12 and 18 months and infant's weight and length at 30 months of age (range 29–38 months of age) were therefore obtained from the 7 health care centers. Infants were consistently weighed completely unclothed on either a Seca model 727 or model 757 scale, (Vogel & Halke, Germany), depending on the health care center. Length was measured with the infant placed supine on a standard measuring board with a sliding foot plate (Seca model 207, Vogel & Halke, Germany) to 18 months of age. After 18 months of age, the children's weight were measured without shoes or socks while standing erect on a Seca model 761 scale (Vogel & Halke, Germany) and their height measured with Seca Body meter 208 (Vogel & Halke, Germany), depending on the health care center. Head circumference was measured with non-stretchable tape at all ages. Infants were measured once at each visit by an experienced child health nurse trained specifically for

the study. All anthropometric measures were converted to z scores using the WHO Child Growth Standards for children aged 0-59 months.<sup>(212)</sup> Cut points for classifying overweight and obesity in study subjects were according to the standards set forth by the WHO. Risk for overweight was defined as BMI-for-age >1 standard deviation (SD), above the WHO growth standard median. Overweight or obese was defined as a value >2 and >3 SDs, respectively, above the WHO growth standard median.<sup>(213)</sup>

#### **4.4 Additional National prospective cohort study**

In paper V in this thesis, infants from a national prospective cohort study conducted in Iceland were included in the data analysis. These infants were born between January and December 2005 and were randomly selected by Statistics Iceland when they were 4 months old. A total of 250 infants were collected in the study. The infants were born in Iceland and inclusion criteria for participation was singleton birth, gestational length at least 37 weeks and absence of congenital abnormalities or chronic health issues.<sup>(214)</sup> Infants in the national prospective cohort study received routine care at the well-baby clinics, however infants in the randomized controlled trial had unlimited access to lactation consultant during the intervention period from 4 to 6 months of age.

##### *4.4.1 Data collection*

Dietary data was collected prospectively monthly from 5-8 months by 24-hour food records, where mothers recorded all foods the infant consumed. These dietary data were used in the analysis in paper V, including dietary data from the randomized controlled trial. Information on total duration of breastfeeding was collected retrospectively for all infants.

#### **4.5 Statistical analysis**

The sample size of 50 mother-infant pairs who completed their participation in each group (a total of 100) was based on primary outcome of the study, breast milk intake. That sample size allowed for the detection of an effect size of 0.6 between the two groups with 5% significance level (two-sided comparison) and 80% power. The sample size calculation was based on the hypothesis that infants in the EBF group would consume more breast milk than infants in the group CF.<sup>(198)</sup> Subjects were recruited until 100 had completed the protocol. Statistical analysis was performed using SPSS software (version 20.0; SPSS INC, Chicago, IL).

For descriptive analysis, data were presented as mean and SD when normally distributed and median and interquartile range (IQR) when not normally distributed and as counts and percentages for categorical variables. For comparison between two groups, the independent t-test was used when data were normally distributed and the non-parametric Mann-Whitney U test was used when data were not normally distributed. Chi-Square tests of

association and two-sided Fisher's exact test was used to compare dichotomous variables. Regression analysis was done to evaluate the impact of intervention group on various health outcomes. If there were baseline characteristics that were significantly different between the two intervention groups, for all subjects or among those who finished the whole trial, they were adjusted for in the regression analysis. In paper V, using the additional national prospective cohort, a one-way ANOVA and Bonferroni Post Hoc Test were used to assess the differences between >2 groups. The level of significance in the study was set at  $p \leq 0.05$ .

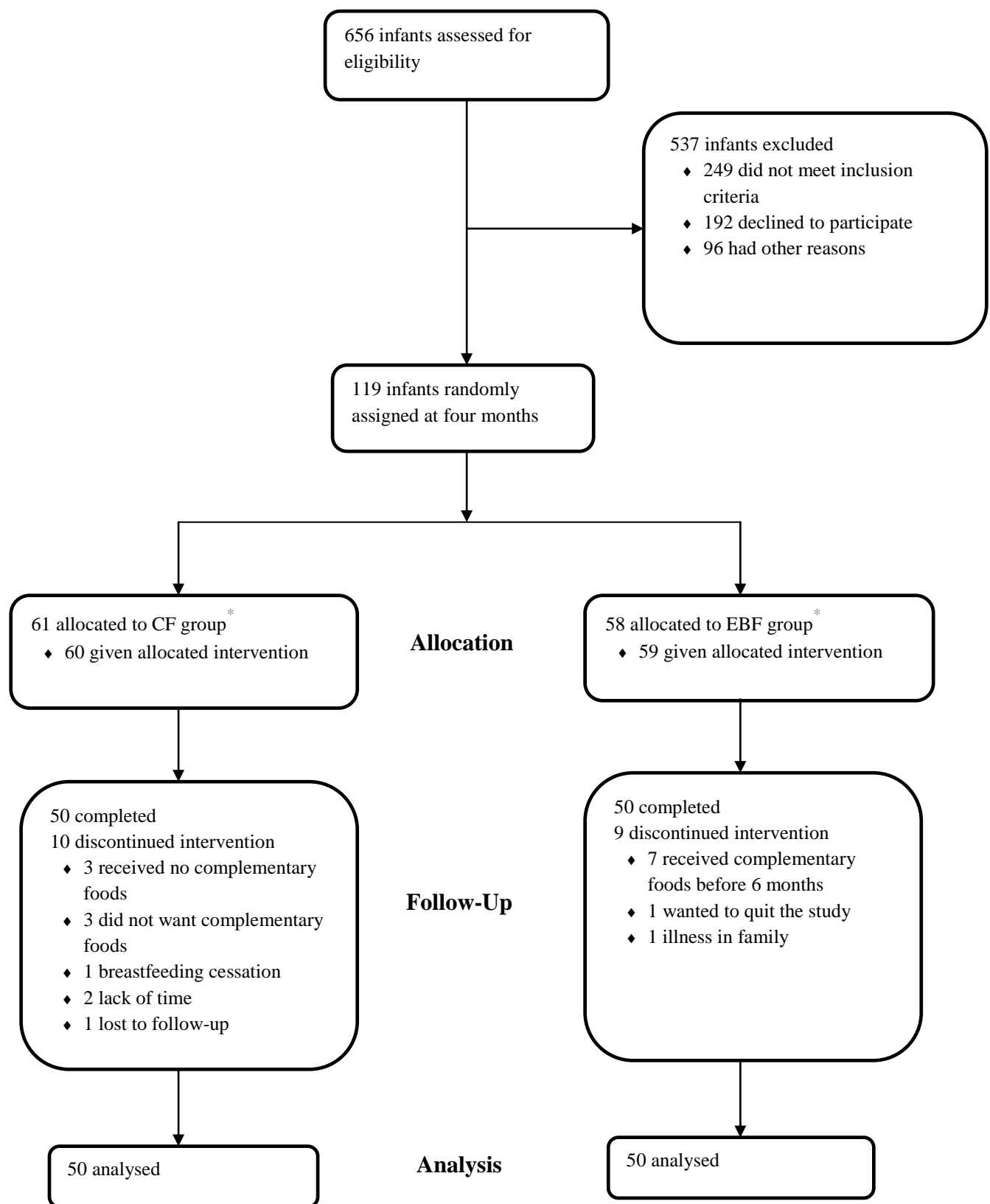
#### **4.6 Approval**

The study was reviewed and approved by the Data Protection Authority and National Bioethical Committee in Iceland and the Partners Health System IRB in Boston, Massachusetts. Informed written consent was obtained from all participants.

## 5. Results and discussion

### 5.1 Baseline characteristics of participants

A total of 119 mother-infant pairs were enrolled in the study between November 2007 and November 2009 (**Figure 4**). A modified per protocol analysis were conducted for the mother-infant pair who was incorrectly instructed to the EBF group, so in the main analyses this infant was analyzed in the EBF group. The outcomes were the same regardless of whether an intent to treat or a modified per protocol analysis was conducted. The dropout rate in the present study was low and similar in both study groups; 10 dropouts in the CF group and 9 in the EBF group. The length-for-age at 4 months was higher in the dropout group than among those who finished the whole intervention trial ( $1.07 \pm 0.59$  vs.  $0.65 \pm 0.86$ ;  $p=0.01$ ); also the dropouts gained length faster from birth to 4 months ( $0.16 \pm 0.90$  vs.  $-0.43 \pm 1.00$ ;  $p=0.02$ ). Thus it is possible that some of those infants who left the study did so because of higher energy demand. Mothers who dropped out of the study had significantly fewer children in the household ( $1.0 \pm 1.0$  vs.  $2.0 \pm 2.0$ ;  $p=0.03$ ). When categorized according to intention to treat no statistically significant differences were seen between groups in baseline characteristics in mothers or infants (**Table 1**). No significant differences were seen between intervention groups in the characteristics (same as shown in Table 1) of subjects who completed the whole trial ( $n=100$ ), except for mode of delivery, where vaginal delivery was more common in the CF group (90% vs. 73% in the EBF group,  $p=0.03$ ).



**Figure 4.** Trial profile: infants who received complementary foods in addition to breast milk from 4 months (CF) compared with infants who were exclusively breastfed for 6 months (EBF). \*One mother-infant pair incorrectly instructed to the EBF group.

**Table 1.** Baseline characteristics of subjects: infants who received complementary foods in addition to breast milk from 4 months (CF) compared with infants who were exclusively breastfed for 6 months (EBF)<sup>\*</sup>, categorized according to intention to treat

	CF (n=61)	EBF (n=58)
<b>Infant characteristics</b>		
Boys	31 (51%) <sup>†</sup>	24 (41%) <sup>†</sup>
Age at randomization (days)	122.9 (3.3)	123.1 (3.1)
Gestational length (days)	281.5 (8.4)	279.6 (9.3)
Weight-for-age at birth <sup>‡</sup>	0.86 (0.83)	0.78 (0.96)
Birth weight (kg)	3.72 (0.43)	3.68 (0.51)
Length-for-age at birth <sup>‡</sup>	1.07 (0.89)	1.03 (1.06)
Length at birth (cm)	51.5 (1.7)	51.4 (2.1)
Head c.-for-age at birth <sup>‡</sup>	1.40 (1.01) <sup>§</sup>	1.44 (1.08)
Head circumference at birth (cm)	35.9 (1.3) <sup>§</sup>	35.9 (1.4)
Weight-for-age at 4 mo <sup>‡</sup>	0.36 (0.98)	0.32 (0.80)
Weight at 4 mo (kg)	7.07 (0.92)	6.97 (0.79)
Length-for-age at 4 mo <sup>‡</sup>	0.68 (0.88)	0.75 (0.80)
Length at 4 mo (cm)	64.5 (2.2)	64.5 (1.9)
Head c.-for-age at 4 mo <sup>‡</sup>	0.93 (0.71)	0.92 (0.88)
Head circumference at 4 mo (cm)	42.3 (1.1)	42.2 (1.3)
<b>Maternal characteristics</b>		
Age at randomization (years)	29.7 (5.2)	30.1 (5.2)
Parity	2.0 (2.0) <sup>  </sup>	2.0 (2.0) <sup>  </sup>
Maternal education at university level <sup>¶</sup>	36 (59%) <sup>†</sup>	29 (50%) <sup>†</sup>
Vaginal delivery	54 (89%) <sup>†</sup>	45 (78%) <sup>†</sup>

Data are mean (SD) unless otherwise indicated

<sup>\*</sup>One mother-infant pair was incorrectly instructed to group EBF

<sup>†</sup>Data are presented as number (%)

<sup>‡</sup>z scores calculated using WHO reference

<sup>§</sup>One missing value, n=60

<sup>||</sup>Data are presented as median (IQR)

<sup>¶</sup>Finished studies at university level

## 5.2 Diet in infancy (Paper I, II)

### 5.2.1 Breast milk intake

Infants of mothers randomized to the EBF group consumed 83 g/d (95% CI 19, 148) more breast milk at 6 months (901±158 g/d in the EBF group and 818±166 g/d in the CF group,

p=0.012). Regression analysis showed that adjustment for parity and mode of birth delivery, the EBF group still consumed significantly more breast milk ( $\Delta=83$  g/d 95% CI 18, 154) than the CF group. The findings for breast milk intake ( $\Delta=76$  g/d 95% CI 11, 141) did not change if the analysis was conducted according to intention to treat. Furthermore, infants in the EBF group consumed slightly more breast milk than the WHO recommendations of  $854\pm24$  g/d ( $\Delta=47$  g/d 95% CI 2, 92), p=0.04). The breast milk intake of infants in the CF group did not significantly differ from the WHO recommendation ( $\Delta=-36$  g/d 95% CI -83, 11).<sup>(10)</sup>

The findings of the present study were similar to those of two trials in Honduras, a population with high frequency of low birth weight, where breast milk intake at 6 months was greater in infants exclusively breastfed for 6 months compared with those receiving complementary foods from 4 months of age in addition to breast milk.<sup>(34, 35)</sup> Measurement of breast milk intake is also challenging, as intakes cannot be studied using approaches common in other age groups. Techniques adapted for infants, such as test-weighing, may intrude on normal behavior of mother and/or infant, and hence bias the results.<sup>(88)</sup> Breast milk intakes measured by isotope studies are objective.

### 5.2.2 *Complementary feeding intake*

The intake of complementary foods by infants in CF group was much lower than expected, with an average energy intake of  $265.4\pm219.0$  kJ/d ( $63.4\pm52.3$  kcal/d) and  $36.8$  kJ/kg body weight ( $8.8$  kcal/kg bodyweight); 10% of the average daily energy requirements for infants 6-11 months of age (**Table 2**). Energy from the complementary foods was 49% attributable to infant cereals, 12% to fruit/vegetables, 18% to infant formula, 13% to fruit purée, 3% from oils or butter, 1% from infant vitamin or cod liver oil and 4% from other foods. The average daily intake of complementary foods among infants in the CF group was  $92.2\pm66.5$  g given as a drink or as part of foods prepared by the parents, such as porridge. The remaining foods comprised 17% infant formula, 16% fruit purée, 12% fruits/vegetables, 9% infant cereal dry mass and small amounts of other foods. Furthermore, the mean daily iron intake from complementary foods was  $0.6$  mg/d at the age of 5 months and 1 week, 8% of an infant's average daily iron requirements at the age of 6-11 months. The main sources of iron from the complementary foods consumed by infants in the CF group were infant cereals (67%), infant formula (17%) and fruit purées (8%). Vitamin C intake from complementary foods mostly came from fruit purée (43%), infant cereals (21%), fruits and vegetables (15%) and infant formula (14%). Iron or vitamin C intake did not correlate with any iron status indices at 6 months, nor did total food or formula intake.

Energy consumption, assuming the metabolizable energy content of breast milk at 6 months to be  $62.1$  kcal/100 g, was similar in both groups ( $560\pm98.2$  kcal/d in the EBF group and  $571\pm97$  kcal/d in the CF group). The similar estimated energy intakes of the two groups indicates that the complementary foods acted primarily to replace breast milk. Average energy intake and the amount of iron that these infants received from complementary foods was small, only 59% of the mean energy intake ( $451$  kJ/d) and 15% of the mean iron intake ( $4.1$  mg/d) that breastfeeding infants in Honduras received from solid foods at 21 weeks of age.

<sup>(215)</sup> This low complementary food intake might be explained by the manner in which the



study was presented to the mothers of infants in the CF group. The mothers were told only to give their infant some complementary foods daily, but no amount was specified. Alternatively, these infants may have consumed more complementary foods during the course of the study than parents recorded, either overall or during the specific 3 day period when they weighed and recorded the foods.

**Table 2.** Intake of selected nutrients received at 5.25 months of age as an average intake from weighed dietary registration of complementary foods (n=50). All infants received breast milk in addition to the complementary foods, breast milk is not included in the table<sup>\*</sup>

	<b>Mean (SD)</b>	<b>Median (IQR)</b>	<b>RDI (6-11 mo)</b>	<b>% of RDI</b>
Energy (kJ/d)	265.4 (219.0)	178.1 (271.0)	-	-
Energy (kJ/kg)	36.8 (32.1)	22.5 (42.2)	355	10
Protein (g/d)	1.3 (1.1)	0.9 (1.3)	-	-
Protein (g/kg)	0.2 (0.2)	0.1 (0.2)	1.1	18
Iron (mg/d)	0.6 (0.5)	0.3 (0.7)	8	8
Vitamin C (mg/d)	8.8 (9.9)	3.9 (15.5)	20	44
Calcium (mg/d)	32.5 (41.7)	11.2 (46.3)	540	6
Vitamin D (µg/d)	4.3 (4.6)	2.5 (9.9)	10	43

<sup>\*</sup>One mother-infant pair was incorrectly instructed to group EBF and was therefore analyzed in group EBF

### 5.3 Anthropometric outcomes in infancy (Paper I, II)

There was no indication of energy inadequacy among infants in the EBF group since no significant differences were seen in growth rate (gain in weight, length and head circumference according to z scores), between the groups prior to the intervention period (0-4 months) or during the intervention period (4-6 months) or from birth to 6 months, categorized according to infants diet during the study period (**Table 3**). Anthropometry z-scores were consistently above zero, and although this partly reflects their large size at birth, typical in the Icelandic population <sup>(216)</sup>, z-scores at 4 and 6 months in the two groups were very similar, as was lean mass at 6 months, giving no indication of growth faltering in the EBF group.

**Table 3.** Growth rate in z scores and body composition at 6 months in the two intervention groups: infants who received complementary foods in addition to breast milk from 4 months (CF) compared with infants who were exclusively breastfed for 6 months (EBF) \*, categorized according to infant diet during the study period

Variables	CF (n=50)	EBF (n=50)	p-value
Weight gain from 0-4 mo <sup>†</sup>	-0.54 (1.01)	-0.42 (1.04)	0.51
Length gain from 0-4 mo <sup>†</sup>	-0.41 (0.85)	-0.26 (1.14)	0.40
Gain in head circumference from 0-4 mo <sup>†</sup>	-0.47 (0.84) <sup>‡</sup>	-0.52 (1.03)	0.80
Weight gain from 4-6 mo	-0.02 (0.31)	-0.01 (0.42)	0.90
Length gain from 4-6 mo	0.03 (0.50)	0.04 (0.51)	0.96
Gain in head circumference from 4-6 mo	0.06 (0.40)	0.06 (0.48)	0.99
Weight gain from 0-6 mo	-0.55 (1.12)	-0.46 (1.17)	0.71
Length gain from 0-6 mo	-0.41 (0.95)	-0.37 (1.18)	0.85
Gain in head circumference from 0-6 mo	-0.36 (0.87) <sup>‡</sup>	-0.41 (1.01)	0.80
Lean mass at 6 mo (kg) <sup>§  </sup>	5.13 (0.92)	4.96 (1.18)	0.40
Fat mass at 6 mo (kg) <sup>§  </sup>	2.71 (0.96)	3.04 (1.12)	0.14

Data are mean (SD) unless otherwise indicated

\* One mother-infant pair was incorrectly instructed to group EBF and was therefore analyzed in group EBF

<sup>†</sup>CF: n=60; EBF: n=59

<sup>‡</sup> One missing value

<sup>§</sup>Isotope measurements

<sup>||</sup>CF: n=43; EBF: n=46

The results of the present study regarding growth are consistent with results from Kramer and Kakuma's systematic review,<sup>(9, 37)</sup> which was the basis of the WHO recommendation regarding the optimal duration of exclusive breastfeeding. The review concluded that there was no reduction in growth in children exclusively breastfed for 4 vs. 6 months. These findings are also consistent with the similar energy intakes seen in the EBF and CF group. Furthermore, data from 2 randomized trials from Honduras showed similar growth rates in both groups and indicated no adverse effect of 6 months of exclusive breastfeeding on growth,<sup>(34, 35)</sup> but whether this applies to populations with larger infant size requires confirmation.

In the present study, we have shown that high proportion (77%) of the mothers who exclusively breastfed for 4 months successfully continued exclusively breastfeeding to 6 months, with their infants showing adequate growth. A longitudinal study, in Glasgow, showed that mothers who were exclusively breastfeeding their infants and were recruited from breastfeeding support groups increased breast milk output between 4 and 6 months.<sup>(85)</sup> The findings of the current study suggest that breast milk alone for 6 months after birth successfully meets energy requirements. Mothers often concern that their breast milk has

become insufficient as a source of nutrition for their infant and that is often a reason for introducing infant formula or complementary foods.<sup>(217, 218)</sup> The evidence on the breast milk intakes of infants exclusively breastfed for 6 months in the current study will hopefully help encourage mothers to exclusively breastfeed.

#### 5.4 Iron status at six months (Paper II)

Blood samples were obtained from 97 infants, where in one case the blood sampling was not successful and also two infants did not show up for the blood sampling. Infants in the CF group had significantly higher SF levels than those in the EBF group ( $p=0.02$ ) (**Table 4**). Excluding one outlier from the data analysis, the infants in the CF group still had significantly higher SF level at 6 months ( $70.0 \pm 73.0 \mu\text{g/l}$  vs.  $43.0 \pm 50.0 \mu\text{g/l}$  in the EBF group;  $p=0.013$ ). Excluding infants ( $n=16$ ) who had any problem that might have affected the ferritin level (infections, C-reactive protein concentration  $>10 \text{ mg/l}$ <sup>(219)</sup> or those in the EBF group who received any complementary foods before 6 months) the difference in SF level remained significant, with higher levels of SF among infants in the CF group ( $67.0 \pm 61.0 \mu\text{g/l}$  vs.  $34.0 \pm 44.5 \mu\text{g/l}$  in the EBF group;  $p=0.003$ ).

**Table 4.** Iron status indices at 6 months in the two intervention groups: infants who received complementary foods in addition to breast milk from 4 months (CF) compared with infants who were exclusively breastfed for 6 months (EBF)<sup>\*</sup>, categorized according to infant diet during the study period

Variables	CF (n=50)	EBF (n=50)	p-value
Hb (g/l) <sup>†</sup>	113.9 (6.1)	113.7 (7.3) <sup>‡</sup>	0.91
MCV (fl) <sup>†</sup>	74.4 (3.3)	73.4 (2.6) <sup>‡</sup>	0.11
SF ( $\mu\text{g/l}$ ) <sup>†</sup>	70.0 (73.3) <sup>§</sup>	44.0 (53.8) <sup>§</sup>	0.02
TIBC ( $\mu\text{mol/l}$ ) <sup>†</sup>	59.2 (9.2)	60.8 (10.1)	0.43
RDW <sup>†</sup>	13.0 (0.7)	13.2 (0.7) <sup>‡§</sup>	0.13

Data are mean (SD) unless otherwise indicated

<sup>\*</sup>One mother-infant pair was incorrectly instructed to group EBF and was therefore analyzed in group EBF

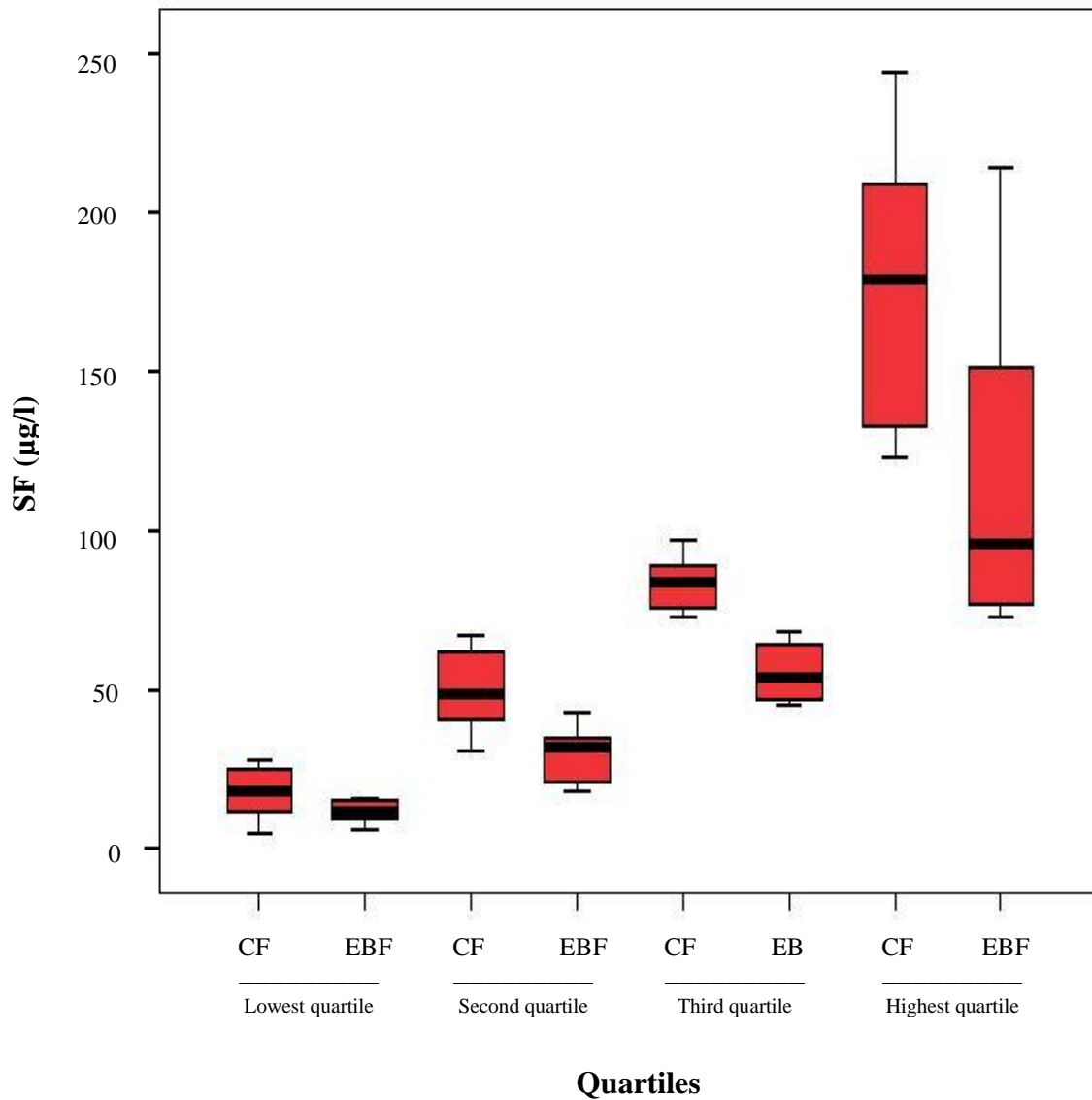
<sup>†</sup>Hb, MCV & RDW  $n=98$ , SF  $n=94$ , TIBC  $n=95$

<sup>‡</sup>Mean (SD) calculated with values from a blood sample taken two weeks later than 6 months from one infant, because some indices were inadvertently not examined on the first sample and were therefore examined on the second sample

<sup>§</sup>Data are median (IQR)

Additionally, comparison of SF between groups were also divided into quartiles and presented as box plots; in each quartile infants who received complementary foods had higher SF levels, significant in all quartiles (**Figure 5**). While infants in the CF group had higher iron stores at 6 months compared to those in the EBF group, both had adequate stores as measured by serum ferritin levels and no significant differences were seen between groups among those who had depleted iron stores (5 in group EBF vs. 2 in group CF;  $p=0.44$ ) or in iron deficiency with anemia (1 in the EBF group vs. 1 in the CF group;  $p=1.00$ ) or without anemia (4 in the EBF group vs. 2 in the CF group;  $p=0.68$ ). There was no significant difference in Hb, MCV, TIBC or RDW between the two study groups. It is controversial which markers should be used to detect iron deficiency. The iron status indicators and the thresholds chosen in the study are in line with studies on infant population in the neighboring countries and previous infant studies conducted in Iceland.

The mode of delivery was significantly different between groups among those who finished the intervention and that could affect the timing of umbilical cord clamping. Delayed cord clamping can have a beneficial effect on infant iron and hematological status through 6 months of age.<sup>(100-102, 106)</sup> After adjustments for mode of delivery, the association of intervention diet with SF was still seen ( $p=0.035$ ), suggesting that the higher iron stores were related to the complementary feeding. These findings are consistent with those of a similar study conducted in a low resource country, where infants who were exclusively breastfed for 6 months had lower measures of iron stores at 6 months compared with infants who received solid foods in addition to breast milk (48.4 vs. 67.3  $\mu\text{g/L}$ ).<sup>(215)</sup> Other investigators have shown that infants with a higher growth rate are at greater risk of ID,<sup>(92, 94)</sup> thought to be accounted for by more rapidly depleted iron stores from birth with greater weight gain.<sup>(95)</sup> Since weight gain did not differ between groups in this study, weight gain cannot account for the differences in the observed serum ferritin levels. In our study we considered that vitamin C in complementary foods could have an effect on iron absorption<sup>(220)</sup> but we saw no relationship between vitamin C intake and measures of iron status. This is likely related to low intakes of complementary foods, and thus both iron and vitamin C, among these infants. These infants may have consumed more complementary foods during the course of the study than parents recorded, either overall or during the specific 3 day period when they weighed and recorded the foods. Nevertheless, infants in the CF group stored significantly more iron. The biological importance of this finding remains to be seen, since both groups had values of serum ferritin considered to be within normal values.



**Figure 5.** Box plot of SF divided into quartiles for comparison between groups in each quartile (one outlier excluded). Mean and standard deviations for each group in each quartile are presented in parentheses: lowest quartile (CF:  $17.8 \pm 8.4$  µg/l vs. EBF:  $11.9 \pm 3.4$  µg/l; n=22; p=0.05), second quartile (CF:  $49.4 \pm 12.7$  µg/l vs. EBF:  $29.9 \pm 8.5$  µg/l; n=25; p<0.001), third quartile (CF:  $83.4 \pm 7.8$  µg/l vs. EBF:  $55.5 \pm 8.6$  µg/l; n=24; p<0.001) and highest quartile (CF:  $176.6 \pm 45.2$  µg/l vs. EBF:  $117.4 \pm 47.5$  µg/l; n=22; p=0.007).

## 5.5 Effects of exclusive breastfeeding for 4 vs. 6 months in early childhood (Paper III, IV)

### 5.5.1 *Developmental and behavioral status*

Since infants exclusively breastfed for 6 months in the present study had significantly higher breast milk intakes at 5.5-6 months of age we hypothesized they would have better developmental and behavioral status in early childhood. A total of 95 children attended the routine care visit at their health care center at 18 months and 82 children at 30-35 months. 54 parents answered the PEDS questionnaire when their child was 18 months and 78 parents at the 30-35 months visit. Since both PEDS questionnaire and Brigance Screens-II were introduced in 2010 there was 41 missing data points from PEDS questionnaire at 18 months of age for those children in the study born in 2007 and some of those born in 2008. Parents of 4 children who attended routine care at 30-35 months did not answer the PEDS questionnaire at that age. The Brigance Screens-II was undertaken by 77 children at the age of 30-35 months, but 11 of them were out of desired age range.

At 18 months a significantly smaller percentage of parents had concerns about any of the domains of PEDS on their children's developmental and behavioral status in the CF group compared with those in the EBF group (17% in group CF vs. 44% in group EBF;  $p=0.03$ ) but the difference was not significant when adjusted for baseline differences ( $p=0.08$ ) (**Table 5**). At 30-35 months, an even smaller proportion of parents in group CF (2%) expressed their concerns about their children's gross motor development in PEDS questionnaire compared with parents of those in group EBF (19%;  $p=0.01$ ), a difference that remained significant when adjusted for baseline differences ( $p=0.03$ ). Use of cut off  $\geq 2$  predictive concerns for PEDS questionnaire at 30-35 months showed that 19% of parents in the EBF group were above cut off value compared with 5% of parents in the CF group, although the difference was not significant ( $p=0.07$ ). Furthermore, there were no significant intergroup differences in developmental total scores ( $p=0.82$ ) or in fine and gross motor skills or receptive and expressive language according to Brigance Screens-II at 30-35 months.

The reason for no difference in these developmental and behavioral measures might be because both study groups consumed a significant amount of breast milk the first 6 months of their lives. While the infants in the EBF group consumed a mean of 83 g/d more of breast milk than those in the CF group, the amount consumed by the CF group was consistent with the recommendations of the WHO.<sup>(10)</sup> Furthermore, a reason for no difference might be because no difference was seen in the prevalence of iron deficiency with or without anemia at 6 months between the study groups. Studies have shown that infants with depleted iron stores, ID or IDA can have lower developmental scores in childhood.<sup>(11, 221)</sup>

In addition to breast milk per se, other factors that influence infant development may have played a role in the outcomes we observed in this randomized trial. Mothers who choose to breastfeed may differ from those who never breastfeed in many ways that can influence an infant's development, including socio-economic status and nurturing qualities. However mothers in both study groups exclusively breastfed for the first 4 months of their infant's life all were from a similar socioeconomic background and thereafter all of them partially or

continued exclusively breastfeeding until 6 months of age or beyond, minimizing the impact of these other influential developmental factors.

**Table 5.** Selected measures of developmental and behavioral status for children at 18 months (PEDS questionnaire: n=29 in group CF, n=25 in group EBF) and at 30–35 months of age (PEDS questionnaire: n=42 in group CF, n=36 in group EBF; Brigance Screens-II: n=35 in group CF, n=31 in group EBF) in the two intervention groups: infants who received complementary foods in addition to breast milk from 4 months (CF) compared with infants who were exclusively breastfed for 6 months (EBF).

Variables	Group CF	Group EBF	p-value
<i>PEDS questionnaire</i>			
Parents with concerns according to PEDS at 18 months	5 (17%)*	11 (44%)*	0.03
Parents with concerns according to PEDS at 30-35 months	14 (33%)*	15 (42%)*	0.45
<i>Brigance Screens-II</i>			
Total score at 30-35 months	86.0 (12.5) <sup>†</sup>	86.5 (12.5) <sup>†</sup>	0.82
Total score above cut off value <sup>‡</sup>	2 (6%)*	4 (13%)*	0.41
Score of predictive factors combined above cut off value <sup>§</sup>	7 (20%)*	3 (10%)* <sup>  </sup>	0.32
Components of the Brigance Screens-II			
Gross motor skills	6.0 (6.0) <sup>†</sup>	6.0 (4.5) <sup>†  </sup>	0.44
Fine motor skills	19.0 (3.0) <sup>†</sup>	19.0 (3.0) <sup>†  </sup>	0.89
Expressive and receptive language	40.5 (8.0) <sup>†</sup>	42.0 (9.5) <sup>†  </sup>	0.81

Data are presented as mean (SD) unless otherwise indicated

\*Data are presented as number (%)

<sup>†</sup>Data are presented as median (IQR)

<sup>‡</sup>Cut off values for defining risk of developmental delay were <72 and <76 points from the total score from the Brigance Screens-II for children aged 30-32 months and 33-35 months, respectively.

<sup>§</sup>Cut off values for defining risk of developmental delay were <34 and <35 points from the predictive components of the Brigance Screens-II combined for children aged 30-32 months and 33-35 months, respectively.

<sup>||</sup>Two missing values, n=29

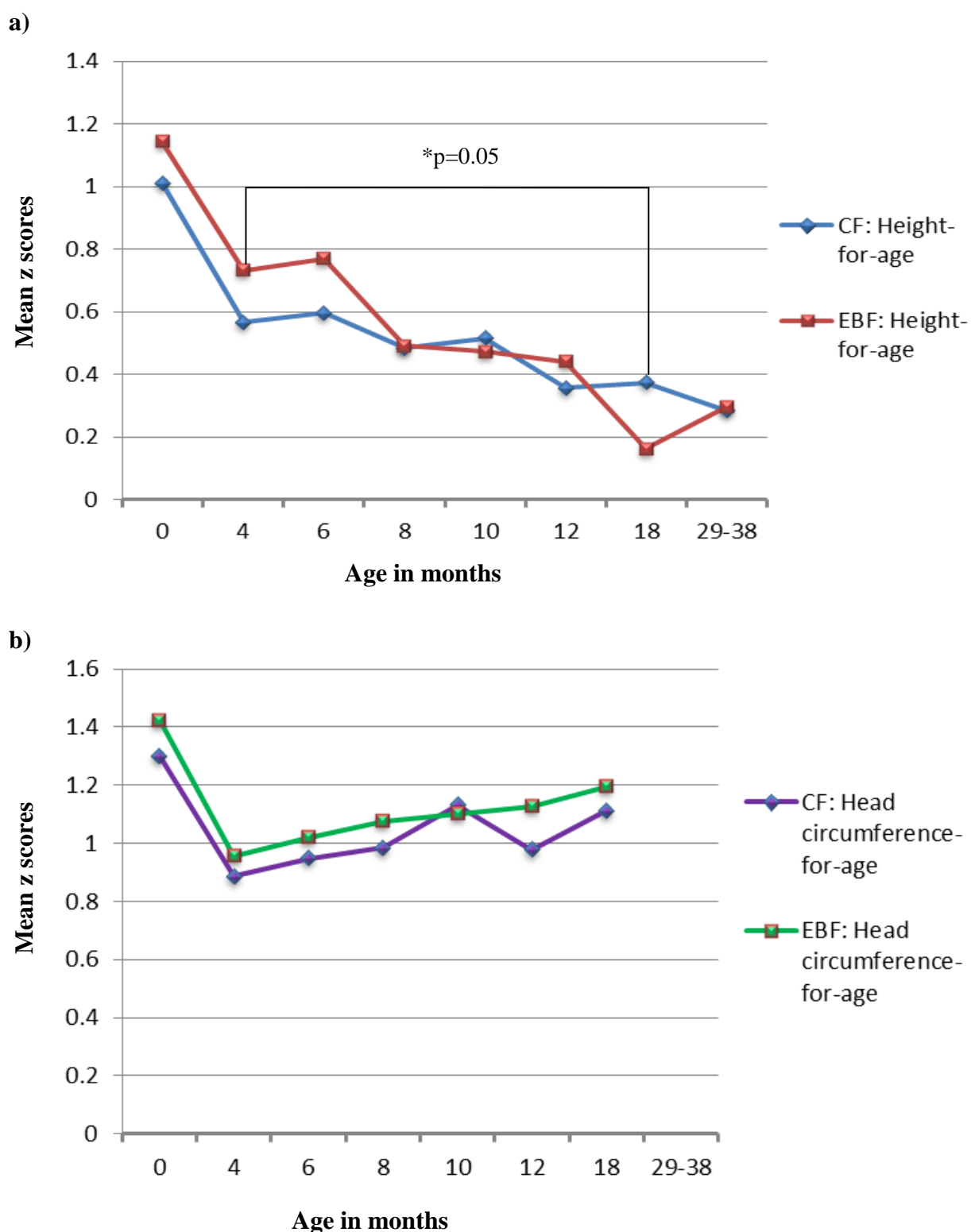
### 5.5.2 Anthropometric outcomes

A total of 97, 96, 98, 95 and 82 infants/children of those 100 who finished the trial attended the routine care visit at their health care center at 8, 10, 12, 18 and 29-38 months,

respectively. There was a missing data points for anthropometric outcomes for 1 child who attended the routine care at 18 months and 5 children at 29-38 months and therefore data was available from 94 and 77 children at these ages. The present study showed a difference in gain in height-for-age according to z scores from the age the intervention started at 4 months of age, and up to 18 months of age, where the gain in length was significantly greater among infants in the CF group ( $-0.17 \pm 0.84$ ,  $n=48$ ) compared with infants in the EBF group ( $-0.51 \pm 0.84$ ,  $n=46$ ;  $p=0.05$ ). Furthermore, faster gain in BMI between 10-12 months was also seen among infants in the CF group ( $0.27 \pm 0.54$ ,  $n=45$ ) compared with those in the EBF group ( $0.06 \pm 0.47$ ,  $n=48$ ;  $p=0.05$ ) and also infants in the CF group had faster weight gain from 10-18 months ( $0.32 \pm 0.48$ ,  $n=46$ ) compared with those in the EBF group ( $0.11 \pm 0.57$ ,  $n = 46$ ), although this was of borderline significance ( $p=0.06$ ). The difference was still significant when adjusted for mode of delivery that was significantly different at baseline. No difference was seen in growth from birth to 4 months, indicating that the intervention from 4–6 months may have affected the growth rate up to 18 months of age but was not detectable at the age of 29–38 months. (**Figure 6, 7**) Furthermore, no effect of longer duration of exclusive breastfeeding were observed on the risk of being overweight ( $>1$  SD) or being overweight ( $>2$  SDs) were observed at 18 and 29-38 months of age according to z scores of BMI-for-age (**Table 6**). The mean BMI z score-for-age for children was  $0.60 \pm 0.92$  and  $0.59 \pm 0.95$  at 18 months for children in the CF group and the EBF group, respectively ( $p=0.96$ ) and  $0.64 \pm 0.86$  and  $0.79 \pm 0.83$  at 29–38 months of age for children in the CF group and the EBF group, respectively ( $p=0.45$ ). Of those infants in the EBF group, 7 mothers gave them formula before 6 months, most of them only once. There was no change in the prevalence of overweight seen among groups by excluding these infants.

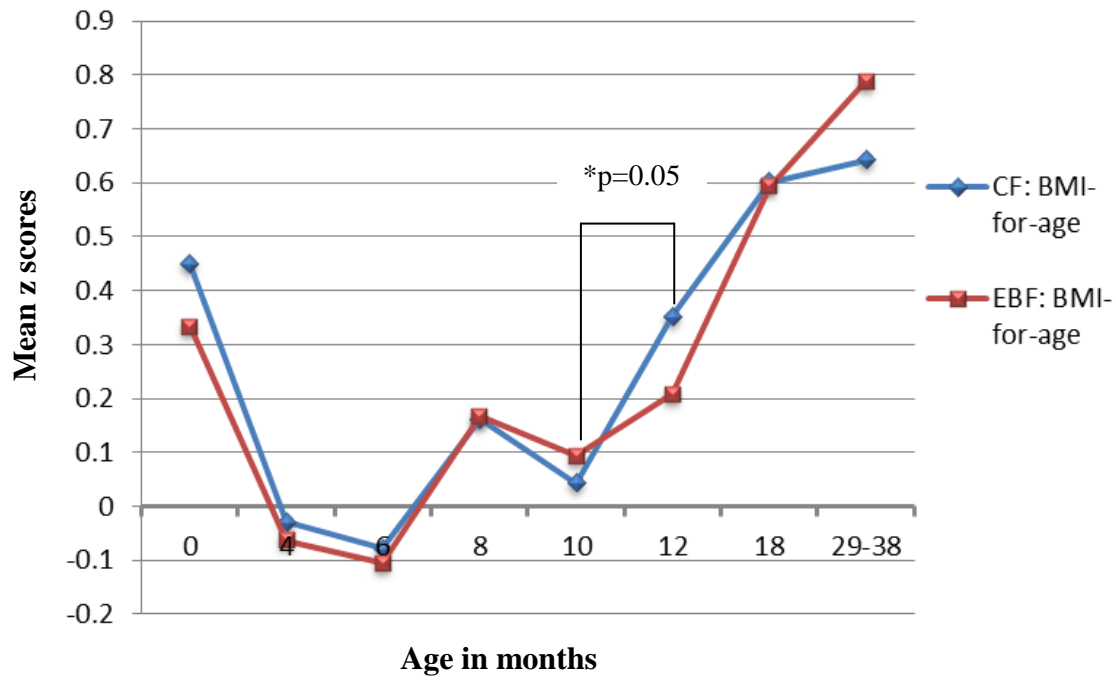
It has been suggested that more rapid weight gain in infancy and childhood may partly be due to the higher protein intakes associated with formula feeding. <sup>(129, 130, 132)</sup> A longer duration of total and exclusive breastfeeding, and therefore lower protein intake, could therefore be protective against later overweight and obesity. <sup>(26, 134-136, 138)</sup> Thus a possible reason for the faster gain in length seen after 4 months in the current study among children in the CF group may be their possible higher protein intakes received from complementary foods between 4 and 6 months of age compared with protein intakes received solely from breast milk in the EBF group. However, this doesn't explain the persistent differences in growth seen to 18 months of age. Unfortunately dietary intake data are not available for these study groups beyond the 4-6 months intervention period. Another possible explanation for the higher growth rates up to 18 months of age seen among children in the CF group may be the theoretical relationship between breastfeeding and development of dietary self-regulation. Some studies suggest that on demand breastfeeding and later introduction of complementary foods may have beneficial effects on self-regulation of energy intake and feeding patterns later in childhood. <sup>(46, 47)</sup> However, in this study, all infants were exclusively breastfed on demand for the first 4 months of life and thereafter breastfed until its termination. The differences in gain in length seen between the two intervention groups during later infancy to 18 months of age remain to be explained and may be a result of differences in dietary intake between groups. The earlier introduction of complementary foods in the CF group did not appear to affect the risk of later overweight or obesity at 18 and 29-38 months of age.



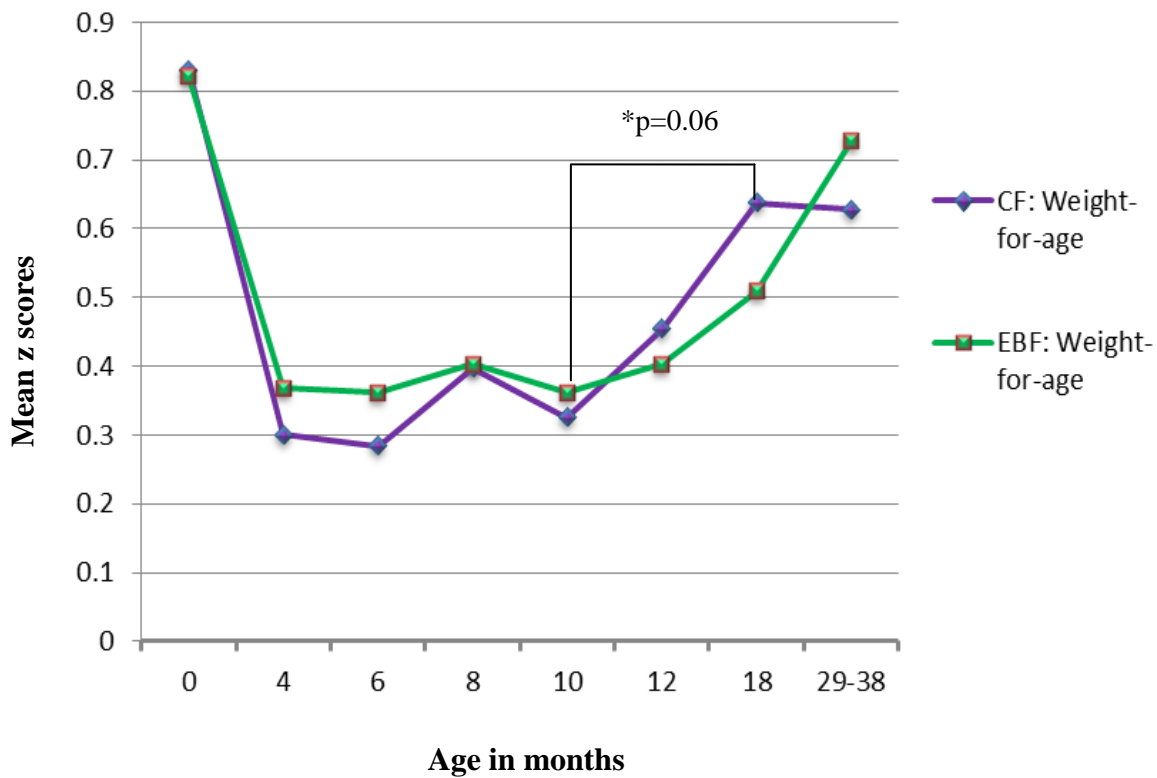


**Figure 6.** Mean height-for-age (a) and mean head circumference-for-age (b) from birth to 29-38 months of age calculated in z scores in the two study groups: infants who received complementary foods from 4 months of age in addition to breast milk (CF) compared with infants who were exclusively breastfed for 6 months (EBF).

a)



b)



**Figure 7.** Mean BMI-for-age (a) and mean weight-for-age (b) from birth to 29-38 months of age calculated in z scores in the two study groups: infants who received complementay foods from 4 months of age in addition to breast milk (CF) compared with infants who were exclusively breastfed for 6 months (EBF).

**Table 6.** BMI-for-age in z scores\* and the prevalence of children at risk of overweight (>1 SD), were overweight (>2 SD) or obese (>3 SD) at 18 months and 29-38 months of age in the two intervention groups: infants who received complementary foods from 4 months of age in addition to breast milk (CF; n=48 at 18 months, n= 40 at 29-38 months) compared with infants who were exclusively breastfed for 6 months (EBF; n=46 at 18 months, n= 37 at 29-38 months).

Variables	Group CF	Group EBF	p-value
<i>18 months of age</i>			
BMI-for-age	0.60±0.92	0.59±0.95	0.96
Risk of overweight	14 (29%) <sup>†</sup>	15 (33%) <sup>†</sup>	0.72
Overweight	4 (8%) <sup>†</sup>	5 (11%) <sup>†</sup>	0.74
Obese	0	0	-
<i>29-38 months of age</i>			
BMI-for-age	0.64±0.86	0.79±0.83	0.45
Risk of overweight	12 (30%) <sup>†</sup>	14 (38%) <sup>†</sup>	0.47
Overweight	4 (10%) <sup>†</sup>	1 (3%) <sup>†</sup>	0.36
Obese	0	0	-

Data are presented as mean (SD) unless otherwise indicated

\* z-scores calculated using the WHO infant growth standards

<sup>†</sup>Number (%)

## 5.6 Effects of breastfeeding support (Paper V)

### 5.6.1 Including data from the additional National prospective cohort study

#### 5.6.1.1 Initial complementary feeding

In the present study a slower introduction of complementary foods, according to number of infant's daily meals at 5 and 6 months of age, was observed among mothers having unlimited access to lactation consultants (**Table 7**) Furthermore, at approximately 5 months of age, a greater proportion of infants whose mothers had unlimited access to lactation consultants consumed some vegetables and vegetable purées over 24 hours ( $p=0.05$ ) and a higher proportion of them had more than one food type in their diet at this age ( $p=0.05$ ) (**Table 8**). Because of the well substantiated advantages of breast milk, recommendations for the introduction of complementary foods of breastfeeding children should take into account the effect of complementary feeding practices on breast milk intake. Some studies suggest that fewer meals and therefore probably higher breast milk intake might be beneficial for infants at this age.<sup>(40, 222)</sup> The difference observed in the present study might be explained by the

intervention itself since those mothers were randomized in either of the two groups and might therefore not see as much benefit of giving complementary foods, especially in big amounts, compared with mothers receiving routine care. Mothers receiving routine care at the well-baby clinics had generally chosen to begin complementary feeding before 6 months for a particular reason, e.g. their infant appeared to be hungry, had trouble sleeping and was waking up more often during the night and might therefore have had more reason for faster introduction of complementary foods. This might weaken the theory that the observed difference in introduction of complementary foods is primarily related to the exposure to lactation consultants. Yet, having easy access to lactation consultants may improve the quality of advice to mothers and give them the confidence to continue to breastfeed, despite encountered problems. Further studies need to be done on the impact of advises by lactation consultants on the initiation of complementary feeding.

**Table 7.** Number of meals at 5 and 6 months of age among infants receiving complementary foods from the age of 4 months in addition to breast milk, having unlimited access to lactation consultant (n=50) or received routine care at the well-baby clinics (n=28).

	5 months of age			6 months of age		
Daily meal frequency	Lactation consultant	Routine care	p-value	Lactation consultant	Routine care	p-value
One meal	34 (68%)	21 (81%)	0.24	18 (37%)	5 (19%)	0.10
Two meals	16 (32%)	3 (12%)	0.06	29 (59%)	11 (41%)	0.12
Three meals	0 (0%)	2 (8%)	0.11	1 (2%)	8 (30%)	0.001
Four meals	0 (0%)	0 (0%)	-	1 (2%)	3 (11%)	0.13

Data are presented as number (%)

<sup>a</sup>Two missing values (n=26)

<sup>b</sup>One missing value (n=49; n=27)

**Table 8.** Selected food types of solid, semi-solid and soft foods infants consumed at approximately 5 months among those who received complementary foods from the age of 4 months in addition to breast milk having unlimited access to lactation consultant (n=50) or received routine care at the well-baby clinics (n=28).

Number of food types	Lactation consultant	Routine care	p-value
Total number of food types	1.5 (1.0) <sup>b</sup>	1.0 (1.0) <sup>b</sup>	0.06
>1 food type	25 (50%)	7 (27%)	0.05
>2 food types	9 (18%)	2 (8%)	0.31
>3 food types	5 (10%)	2 (8%)	1.00
Porridge	40 (80%)	24 (92%)	0.20
>1 type	3 (6%)	2 (8%)	1.00
Vegetables & vegetable purées	11 (22%)	1 (4%)	0.05
>1 type	3 (6%)	0 (0%)	0.55
Fruit & fruit purées	17 (34%)	7 (27%)	0.53
>1 type	4 (8%)	1 (4%)	0.66

Data are presented as number (%) unless otherwise indicated

<sup>a</sup>Two missing values (n=26)

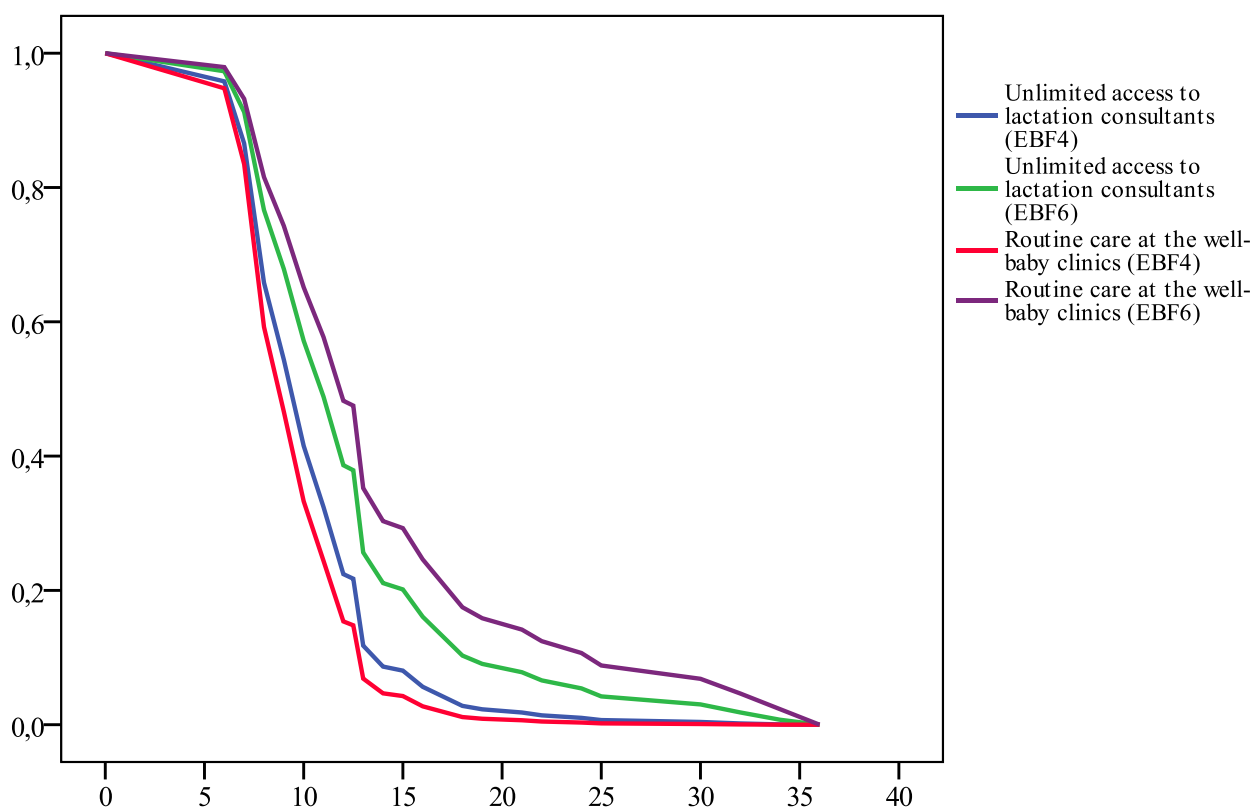
<sup>b</sup>Data are presented as median (IQR)

#### 5.6.1.2 Total breastfeeding duration

Total breastfeeding duration was logarithmically transformed because of its skewed distribution and it differed significantly between the 4 study groups,  $F(3, 133)=5.26$ ,  $p=0.002$ . Bonferroni post-hoc comparisons indicated that infants exclusively breastfed for 6 months whose mothers had unlimited access to lactation consultants ( $1.08 \pm 0.15$ ;  $n=50$ ) had significantly longer total breastfeeding duration than infants who received complementary foods from 4 months of age, both among those who received routine care at the well-baby clinics ( $0.96 \pm 0.13$ ;  $n=26$ , 95% CI 0.03, 0.22) and those who had unlimited access to lactation consultants ( $1.01 \pm 0.11$ ;  $n=49$ , 95% CI -0.001, 0.16). Additionally, no difference was seen in total duration of breastfeeding between the two study groups whose mothers exclusively breastfed their infants for 6 months ( $1.08 \pm 0.27$ ;  $n=12$ , 95% CI -0.12, 0.14) (**Figure 8**).

Results from the present study indicate that exclusive breastfeeding for 6 months appears to support longer total breastfeeding duration compared to exclusive breastfeeding for 4 months, regardless of exposure to lactation consultants. The prevalence rate of exclusive breastfeeding for 6 months is low globally, both in low resource and resource rich countries. <sup>(223)</sup> It is possible that more exposure to lactation consultants the first months of infant's life would increase the prevalence of mothers exclusively breastfeeding for 6 months, and according to the results of the current study, may therefore have more prolonged breastfeeding. While the health benefits of breastfeeding are well known <sup>(9, 16)</sup> less is known about the benefits of prolonged breastfeeding beyond one year of age. More prolonged breastfeeding has been

shown to be associated with a lower incidence of childhood infections. <sup>(5, 19, 224)</sup> It remains to be established whether a longer duration of breastfeeding is associated with a decreased risk of overweight and obesity in childhood and beyond. <sup>(190, 194, 225-227)</sup> Since exclusive breastfeeding for 6 months can increase the total breastfeeding duration compared with infants exclusively breastfed for 4 months, this may have significant benefits beyond the period of infancy.



**Figure 8.** Breastfeeding frequency at every age in the two study populations i.e. infants whose mothers had unlimited access to lactation consultants (received complementary foods at 4 months, EBF4: n=49; exclusively breastfed for 6 months, EBF6: n=50) and those who received routine care at the well-baby clinics (received complementary foods at 4 months, EBF4: n=26; exclusively breastfed for 6 months, EBF6: n=12).

## **6. Strengths and limitations**

In 2001, the WHO recommended and requested randomized trials of exclusive breastfeeding in both low resource and resource rich countries, since previous studies investigating the effects of diet in infancy were mainly observational and therefore not able to demonstrate causality. To our knowledge this is the first and only randomized controlled trial to examine the effects of exclusive breastfeeding for 4 vs. 6 months on various health outcomes in infancy and early childhood in a resource rich country. The principle strength of the present study comprises its randomized trial design of exclusively breastfed infants. Additional strengths of the present study include the use of an objective method for measuring breast milk intake and body composition with stable isotope probes and also conducting the study in Iceland, where the national health system is superbly organized to collect data on infant and child health and the environment is supportive of breastfeeding.

While the sample size may be considered a limitation of the present study, it was intended to serve as a pilot study that would lead to larger replication cohorts. In addition there was a significant difference between the study groups at baseline in mode of delivery. However the difference was relatively minor and even after statistical adjustment, did not account for our main findings regarding breast milk or complementary feeding intakes, iron status, anthropometric outcomes or developmental and behavioral status, except for number of parents' concerns about their infant's development at 18 months. Although conducting the study in Iceland represents a strength, it also represents a limitation as the cold environment and the relatively high birth weights are not typical of most European populations and that might reduce the generalizability of the findings.

Finally, the absence of data on the timing of cord clamping or the iron status of the infants at baseline was a limitation of the study. In Iceland, the timing of cord clamping is not routinely collected at delivery, and we did not have the resources available to collect this information as part of the study, since it would have entailed recruiting a large number of women antenatally who were subsequently not eligible for the trial as they did not fulfill the breastfeeding criteria at 4 months. We also considered that asking to collect blood samples at both 4 and 6 months would likely deter mothers from participation.

## **7. Conclusions**

The results of this thesis indicate that infants exclusively breastfed for 4 and 6 months from birth have similar energy intakes around 5.5 months to 6 of age and exclusive breastfeeding for 6 months does therefore not compromise body composition in infancy or growth in infancy and early childhood. In a resource rich country, adding a small amount of complementary foods in addition to breast milk from 4 months of age has a small and positive effect on iron status at 6 months, however the biological significance of this finding remains to be determined.

Follow-up of the infants in the study showed that no sustained effect of longer duration of exclusive breastfeeding was seen on selected measures of developmental or behavioral status in early childhood. However a smaller percentage of parents of children introduced to complementary foods at 4 months of age expressed concerns about their gross motor development in early childhood. Furthermore, duration of exclusive breastfeeding for 4 or 6 months does not seem to affect the risk of being overweight or the prevalence of those who were overweight in early childhood.

Exposure to lactation consultants may influence feeding behaviors during the initial complementary feeding period and enhance slower introduction of complementary feedings. Furthermore, exclusive breastfeeding for 6 months may result in more prolonged breastfeeding compared with exclusive breastfeeding for 4 months. Mothers with more exposure to lactation consultants might become more confident and likely to follow advice given about complementary feeding compared with those receiving routine care at the well-baby clinics. Further studies are needed to determine the generalizability of these findings and their long term effect on the dietary patterns and food choices of young children.

It is acknowledged that while the reported group effects are significant, feeding must be tailored to meet the needs of the individual infant.



## **8. Future perspectives**

The current study does not determine whether the observed difference in iron status between study groups persists later in infancy and in early childhood and whether later differences are seen in rates of ID or IDA. The sample size of the present study was relatively small and therefore a larger randomized controlled trial with longitudinal data is needed to rule out the long term effects of this early difference of iron status.

Furthermore, it would be interesting to measure the metabolisable energy content of breast milk at 6 months following exclusive breastfeeding to observe if the energy in breast milk is adequate for energy requirements of infants at 6 months. Objective data on this could be provided with stable isotope probes using the "double-labeled water" technique.

The immediate importance of adequate diet in infancy has long been recognized and increasingly its possible effects on health later in life. Both the length of exclusive breastfeeding and the timing of the introduction of complementary feeding needs to be further investigated to determine their long term effect on appetite control, novel food acceptance, health and dietary patterns in childhood. Therefore a larger randomized controlled trial investigating the effects of infant nutrition during the first 6 months on various health outcomes in infancy and childhood is of great importance.

## 9. Acknowledgements

The present work was carried out at the Unit for nutrition research, Faculty of Food Science and Nutrition, School of Health Science, University of Iceland and Landspítali National-University Hospital. Funding was provided by the Mead Johnson Fund, the Eimskip Fund of the University of Iceland and the Icelandic Public Health Fund.

I would never have been able to complete this huge task on my own so in the following paragraphs I would like to express gratitude to all the people who played a part.

First I would like to thank my hard working supervisor, Inga Þórsdóttir, for her guidance and enormous support throughout this journey. Furthermore I would like to thank Alfons Ramel and Þórhallur Ingi Halldórsson for their wise answers to my (often stupid) questions about statistics. Special thanks to Tinna Eysteinsdóttir and Margrét Þóra Jónsdóttir, my sister, for carrying out the intervention while I was on maternity leave after the birth of my older daughter. All my co-workers at the Unit for nutrition research and the team of dieticians at Landspítali National-University Hospital also receive my gratitude for making the work so much more fun.

I am deeply thankful to the nurses Alma M. Rögnvaldsdóttir, Auður Egilsdóttir, Dagný Guðmundsdóttir, Elín Sigurbjörnsdóttir, Guðrún Guðmundsdóttir, Ingibjörg Eiríksdóttir, Kristín J. Vigfúsdóttir, Jóna Margrét Jónsdóttir and Sesselja Guðmundsdóttir for their tremendous part in collecting data during the study and their invaluable assistance throughout this work. I would furthermore like to thank my co-authors of the papers who are all outstanding scientists; Geir Gunnlaugsson, Jonathan C. Wells, Mary S. Fewtrell, Patricia L. Hibberd and Ronald E. Kleinman for providing their useful contributions and always responding quickly to my numerous e-mails. Special thanks to Patricia L. Hibberd and Ronald E. Kleinman for being extremely helpful during the last couple of months and proofreading my thesis. All the co-authors served me incredibly well throughout this work. My endless gratitude goes to the participants of the study for without their contribution the research could not have been conducted.

I am forever grateful for my co-workers Ása Vala, Cindy, Óla Kallý, Svandís and Tinna who became my good friends during my studies; they definitely made all of this worth it. They were always willing to help and listen to me complaining when I felt I would never finish all the papers. They all are and will always be my favourite co-workers and good friends. Just to point out, you are now categorized as friends, not only as co-workers! Ása Vala deserves special thanks for answering my numerous questions about my PhD thesis and Svandís for discovering the residual plot.

My siblings, friends and my fiancé's family of course receive endless gratitude for their love and support throughout this work and their many hours of babysitting in my absence. I am also ever so grateful for their willingness and patience to listen to my endless ramblings about the process of my thesis, even though it was definitely not their favourite topic of discussion! I can hardly describe how grateful I am to my parents, Jón and Sigurveig, for all their love and assistance, they certainly made my life easier during these stressful last couple of months.

Last but not least I am forever grateful to Elvar, my fiancé, for his untiring encouragement and great patience throughout this work and for coping with me when I was stressed and cranky at times. During my PhD studies I gave birth to my two daughters, Aldís Ösp and Sigurveig Sara. The experience of becoming a mother made this study even more relevant to me as I realized just how important successful breastfeeding is for both mother and child. My deepest gratitude goes to them simply for making me smile every single day. They are my greatest achievements in life.

Reykjavik, February 2013

Ólöf Helga Jónsdóttir

## 10. References

1. Breastfeeding and infant nutrition among infants born 2004-2008 in Iceland. Directorate of Health, 2012. Available at [http://www.landlaeknir.is/servlet/file/store93/item16573/version4/brjostagjof\\_og\\_naring\\_2004-2008\\_juni.2012.pdf](http://www.landlaeknir.is/servlet/file/store93/item16573/version4/brjostagjof_og_naring_2004-2008_juni.2012.pdf) Accessed October 31<sup>st</sup>, 2012.
2. World Health Organisation (WHO). Feeding and nutrition in infants and young children. Guidelines for the WHO European Region, with emphasis on the former Soviet countries. 2000. WHO Regional Publications, European Series, No. 87. Denmark.
3. Lucas A. Programming by early nutrition in man. Ciba Found Symp. 1991;156:38–50.
4. World Health Organization (WHO). Nutrition: Information and attitudes among health personnel about early infant-feeding practices. WHO Wkly Epidem Rec. 1995; 70(17):117-20.
5. Kramer MS, Chalmers B, Hodnett ED et.al. Promotion of Breastfeeding Intervention Trial (PROBIT): A Randomized Trial in the Republic of Belarus. JAMA. 2001;285(4):413-20.
6. Monterrosa EC, Frongillo EA, Vásquez-Garibay EM, Romero-Velarde E, Casey LM, Willows ND. Predominant breast-feeding from birth to six months is associated with fewer gastrointestinal infections and increased risk for iron deficiency among infants. J Nutr. 2008;138(8):1499-504.
7. Kramer MS, Guo T, Platt RW et al. Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breastfeeding. Am J Clin Nutr. 2003;78(2):291-5.
8. Fewtrell MS, Morgan JB, Duggan C, Gunnlaugsson G, Hibberd PL, Lucas A, Kleinman RE. Optimal duration of exclusive breastfeeding: what is the evidence to support current recommendations? Am J Clin Nutr. 2007;85(2):635S-638S.
9. World Health Organization (WHO). The optimal duration of exclusive breastfeeding: A systematic review. 2001 WHO/NHD/01.08. Geneva, Switzerland.
10. Butte NF, Lopez-Alarcon MG, Garza C. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. 2002. Geneva, World Health Organization.
11. Gunnarsson BS, Thorsdottir I, Palsson G, Gretarsson SJ. Iron status at 1 and 6 years versus developmental scores at 6 years in a well-nourished affluent population. Acta Paediatr. 2007;96(3):391-5.

12. Griffin IJ, Abrams SA. Iron and breastfeeding. *Pediatr Clin North Am.* 2001;48(2):401-13.
13. Benton D, ILSI Europe a.i.s.b.l. Micronutrient status, cognition and behavioural problems in childhood. *Eur J Nutr.* 2008;47(3):38-50.
14. Georgieff MK. Long-term brain and behavioral consequences of early iron deficiency. *Nutr Rev.* 2011;69(1):S43-8.
15. Aggett PJ, Agostoni C, Axelsson I, Bresson JL, Goulet O, Hernell O, Koletzko B, Lafeber HL, Michaelsen KF, Micheli JL, Rigo J, Szajewska H, Weaver LT. Iron metabolism and requirements in early childhood: do we know enough?: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr.* 2002;34(4):337-45.
16. Section on Breastfeeding. Breastfeeding and the use of human milk. *Pediatrics.* 2012;129(3):e827-41.
17. Schack-Nielsen L, Michaelsen KF. Advances in our understanding of the biology of human milk and its effects on the offspring. *J Nutr.* 2007;137(2):503S-510S.
18. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T, Lau J. Breastfeeding and maternal and infant health outcomes in developed countries. *Evid Rep Technol Assess (Full Rep).* 2007;153:1-186.
19. Duijts L, Ramadhani MK, Moll HA. Breastfeeding protects against infectious diseases during infancy in industrialized countries. A systematic review. *Matern Child Nutr.* 2009;5(3):199-210.
20. Horta BL, Bahl R, Martinés JC, Victora CG. Evidence on the long-term effects of breastfeeding: Systematic reviews and meta-analyses. 2007. Geneva, Switzerland: World Health Organization (WHO).
21. Taylor JS, Kacmar JE, Nothnagle M, Lawrence RA. A systematic review of the literature associating breastfeeding with type 2 diabetes and gestational diabetes. *J Am Coll Nutr.* 2005;24(5):320-6.
22. Patelarou E, Girvalaki C, Brokalaki H, Patelarou A, Androulaki Z, Vardavas C. Current evidence on the associations of breastfeeding, infant formula, and cow's milk introduction with type 1 diabetes mellitus: a systematic review. *Nutr Rev.* 2012;70(9):509-19.
23. Jain A, Concato J, Leventhal JM. How good is the evidence linking breastfeeding and intelligence? *Pediatrics.* 2002;109(6):1044-53.
24. Dewey KG, Cohen RJ, Rivera LL, Canahuati J, Brown KH. Effects of age at introduction of complementary foods to breast-fed infants on duration of lactational amenorrhea in Honduran women. *Am J Clin Nutr.* 1997;65(5):1403-9.

25. Dewey KG. Impact of breastfeeding on maternal nutritional status. *Adv Exp Med Biol.* 2004;554:91-100.
26. Gillman MW, Rifas-Shiman SL, Camargo CA Jr, Berkey CS, Frazier AL, Rockett HR, Field AE, Golditz GA. Risk of overweight among adolescents who were breastfed as infants. *JAMA.* 2001;285(19):2461-7.
27. Ellestad-Sayed J, Coodin FJ, Dilling LA, Haworth JC. Breast-feeding protects against infection in Indian infants. *Can Med Assoc J.* 1979;120(3):295-8
28. Quigley MA, Kelly YJ, Sacker A. Breastfeeding and hospitalization for diarrheal and respiratory infection in the United Kingdom Millennium Cohort Study. *Pediatrics.* 2007;119(4):e837-42.
29. Paricio Talayero JM, Lizán-Garcia M, Otero Puime A, Benlloch Muncharaz MJ, Beseler Soto B, Sánchez-Palomares M, Santos Serrano L, Rivera LL. Full breastfeeding and hospitalization as a result of infections in the first year of life. *Pediatrics.* 2006;118(1):e92-9.
30. World Health Organization (WHO). Infant feeding the physiological basis. *Bulletin of the World Health Organization*, vol. 67, 1989. Available at [http://whqlibdoc.who.int/bulletin/1989/Vol67-Supp/bulletin\\_1989\\_67\(supp\)\\_3.pdf](http://whqlibdoc.who.int/bulletin/1989/Vol67-Supp/bulletin_1989_67(supp)_3.pdf) Accessed November 19<sup>th</sup>, 2012.
31. World Health Organization (WHO). Infant and young child feeding – model chapter for textbooks. 2009. Available at [http://whqlibdoc.who.int/publications/2009/9789241597494\\_eng.pdf](http://whqlibdoc.who.int/publications/2009/9789241597494_eng.pdf) Accessed November 18<sup>th</sup>, 2012.
32. World Health Organization (WHO). Global Strategy on Infant and Young Child Feeding. 55<sup>th</sup> World Health Assembly. 2002. Geneva, Switzerland.
33. World Health Organization (WHO). The Optimal Duration of Exclusive Breastfeeding: Report of an Expert Consultation. 2001. Geneva, Switzerland.
34. Cohen RJ, Brown KH, Canahuati J, Rivera LL, Dewey KG. Effects of age of introduction of complementary foods on infant breast milk intake, total energy intake, and growth: a randomized intervention study in Honduras. *Lancet.* 1994;344(8918):288-93.
35. Dewey KG, Cohen RJ, Brown KH, Rivera LL. Age of introduction of complementary foods and growth of term, low-birthweight, breast-fed infants: a randomized intervention study in Honduras. *Am J Clin Nutr.* 1999;69(4):679-86.
36. Arifeen S, Black RE, Antelman G, Baqui A, Caulfield L, Becker S. Exclusive breastfeeding reduces acute respiratory infection and diarrhea deaths among infants in Dhaka slums. *Pediatrics.* 2001;108(4):E67.

37. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev.* 2012;8:CD003517.
38. Morrow AL, Rangel JM. Human milk protection against infectious diarrhea: implications for prevention and clinical care. *Semin Pediatr Infect Dis.* 2004;15(4):221-8.
39. Lanigan JA, Bishop JA, Kimber AC, Morgan J. Systematic review concerning the age of introduction of complementary foods to the healthy full-term infant. *Eur J Clin Nutr.* 2001;55(5):309-20.
40. The Pan American Health Organization (PAHO). Guiding principles for complementary feeding of the breastfed child. Washington, D.C.: Pan American Health Organization, World Health Organization, 2003. Available at <http://whqlibdoc.who.int/paho/2003/a85622.pdf> Accessed November 18<sup>th</sup>, 2012.
41. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B, Michaelsen KF, Moreno L, Puntis J, Rigo J, Shamir R, Szajewska H, Turck D, van Goudoever J; ESPGHAN Committee on Nutrition. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr.* 2008;46(1):99-110.
42. Caroli M, Mele RM, Tomaselli MA, Cammisa M, Longo F, Attolini E. Complementary feeding patterns in Europe with a special focus on Italy. *Nutr Metab Cardiovasc Dis.* 2012;22(10):813-8.
43. Lande B, Andersen LF, Baerug A, Trygg KU, Lund-Larsen K, Veierød MB, Bjørneboe GE. Infant feeding practices and associated factors in the first six months of life: the Norwegian infant nutrition survey. *Acta Paediatr.* 2003;92(2):152-61.
44. Colin WB, Scott JA. Breastfeeding: reasons for starting, reasons for stopping and problems along the way. *Breastfeed Rev.* 2002;10(2):13-9.
45. Wijndaele K, Lakshman R, Landsbaugh JR, Ong KK, Ogilvie D. Determinants of early weaning and use of unmodified cow's milk in infants: a systematic review. *J Am Diet Assoc.* 2009;109(12):2017-28.
46. Abraham EC, Godwin J, Sherriff A, Armstrong J. Infant feeding in relation to eating patterns in the second year of life and weight status in the fourth year. *Public Health Nutr.* 2012;15(9):1705-14.
47. Fisher JO, Birch LL, Smiciklas-Wright H, Picciano MF. Breast-feeding through the first year predicts maternal control in feeding and subsequent toddler energy intakes. *J Am Diet Assoc.* 2000;100(6):641-6.
48. Grummer-Strawn LM, Scanlon KS, Fein SB. Infant feeding and feeding transitions during the first year of life. *Pediatrics.* 2008;122(2):S36-42.

49. Berenson GS. Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study. *Am J Cardiol.* 2002;90(10C):3L-7L.
50. Caballero B. Early nutrition and risk of disease in the adult. *Public Health Nutr.* 2001;4(6A):1335-6.
51. Lanigan J, Singhal A. Early nutrition and long-term health: a practical approach. *Proc Nutr Soc.* 2009;68(4):422-9.
52. Arabi M, Frongillo EA, Avula R, Mangasaryan N. Infant and young child feeding in developing countries. *Child Dev.* 2012;83(1):32-45.
53. Michaelsen KF, Larsen PS, Thomsen BL, Samuleson G. The Copenhagen cohort study on infant nutrition and growth: duration of breast feeding and influencing factors. *Acta Paediatr.* 1994;83(6):565-71.
54. Rogers IS, Emmett PM, Golding J. The incidence and duration of breast feeding. *Early Hum Dev.* 1997;49:S45-74.
55. Thorsdottir I, Gunnarsdottir I, Kvaran MA, Gretarsson SJ. Maternal body mass index, duration of exclusive breastfeeding and children's developmental status at the age of 6 years. *Eur J Clin Nutr.* 2005;59(3):426-31.
56. Jackson DA, Imong SM, Wongsawasdi L, Silprasert A, Preunglampoo S, Leelapat P, Drewett RF, Amatayakul K, Baum JD. Weaning practices and breast-feeding duration in Northern Thailand. *Br J Nutr.* 1992;67(2):149-64.
57. Yoon PW, Black RE, Moulton LH, Becker S. Effect of not breastfeeding on the risk of diarrheal and respiratory mortality in children under 2 years of age in Metro Cebu, The Philippines. *Am J Epidemiol.* 1996;143(11):1142-8.
58. Brush G, Harrison GA, Zumrawi FY. A path analysis of some determinants of infant growth in Khartoum. *Ann Hum Biol.* 1993;20(4):381-7.
59. Blomquist HK, Jonsbo F, Serenius F, Persson LA. Supplementary feeding in the maternity ward shortens the duration of breast feeding. *Acta Paediatr.* 1994;83(11):1122-6.
60. Oddy WH, Li J, Landsborough L, Kendall GE, Henderson S, Downie J. The Association of Maternal Overweight and Obesity with Breastfeeding Duration. *J Pediatr.* 2006;149(2):185-91.
61. Dubois L, Girard M. Social determinants of initiation, duration and exclusivity of breastfeeding at the population level: the results of the Longitudinal Study of Child Development in Quebec (ELDEQ 1998-2002). *Can J Public Health.* 2003;94(4):300-5.



62. Hörnell A, Aarts C, Kylberg E, Hofvander Y, Gebre-Medhin M. Breastfeeding patterns in exclusively breastfed infants: a longitudinal prospective study in Uppsala, Sweden. *Acta Paediatr.* 1999;88(2):203-11.
63. Haughton J, Gregorio D, Pérez-Escamilla R. Factors associated with breastfeeding duration among Connecticut Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) participants. *J Hum Lact.* 2010;26(3):266-73.
64. Thorisdottir AV, Gunnarsdottir I, Thorsdottir I. Revised infant dietary recommendations: the impact of maternal education and other parental factors on adherence rates in Iceland. *Acta Paediatr.* 2013;102(2):143-8.
65. Gudnadottir M, Gunnarsson BS, Thorsdottir I. Effects of sociodemographic factors on adherence to breastfeeding and other important infant dietary recommendations. *Acta Paediatr.* 2006;95(4):419-24.
66. Meedya S, Fahy K, Kable A. Factors that positively influence breastfeeding duration to 6 months: a literature review. *Women Birth.* 2010;23(4):135-45.
67. Callen J, Pinelli J. Incidence and duration of breastfeeding for term infants in Canada, United States, Europe, and Australia: a literature review. *Birth.* 2004;31(4):285-92.
68. Nordic Expert Group for Nutritional Recommendations: Nordic Nutrition Recommendation 2004. Nordic Council of Ministers, Copenhagen, Nord 2004:13.
69. Wilson AC, Forsyth JS, Greene SA, Irvine L, Hau C, Howie PW. Relation of infant diet to childhood health: seven year follow up of cohort of children in Dundee infant feeding study. *BMJ.* 1998;316(7124):21-5.
70. Bolling K, Grant C, Hamlyn C, Thornton A. Infant feeding survey 2005: The Information Centre; 2007.
71. James DC, Lessen R; American Dietetic Association. Position of the American Dietetic Association: promoting and supporting breastfeeding. *J Am Diet Assoc.* 2009;109(11):1926-42.
72. WHO Multicentre Growth Reference Study Group. Breastfeeding in the WHO Multicentre Growth Reference Study. *Acta Paediatr Suppl.* 2006;450:16-26.
73. Barros FC, Semer TC, Tonioli Filho S, Tomasi E, Victora CG. The impact of lactation centres on breastfeeding patterns, morbidity and growth: a birth cohort study. *Acta Paediatr.* 1995;84(11):1221-6.
74. Vari PM, Camburn J, Henly SJ. Professionally mediated peer support and early breastfeeding success. *J Perinat Educ.* 2000;9(1):22-30.

75. Albernaz E, Victora CG, Haisma H, Wright A, Coward WA. Lactation counseling increases breast-feeding duration but not breast milk intake as measured by isotopic methods. *J Nutr.* 2003;133(1):205-10.
76. Albernaz E, Giugliani ER, Victora CG. Supporting breastfeeding: a successful experience. *J Hum Lact.* 1998;14(4):283-5.
77. de Oliveira MI, Camacho LA, Tedstone AE. Extending breastfeeding duration through primary care: a systematic review of prenatal and postnatal interventions. *J Hum Lact.* 2001;17(4):326-43.
78. Thurman SE, Allen PJ. Integrating lactation consultants into primary health care services: are lactation consultants affecting breastfeeding success? *Pediatr Nurs.* 2008;34(5):419-25.
79. Castrucci BC, Hoover KL, Lim S, Maus KC. A comparison of breastfeeding rates in an urban birth cohort among women delivering infants at hospitals that employ and do not employ lactation consultants. *J Public Health Manag Pract.* 2006;12(6):578-85.
80. Bonuck KA, Trombley M, Freeman K, McKee D. Randomized, controlled trial of a prenatal and postnatal lactation consultant intervention on duration and intensity of breastfeeding up to 12 months. *Pediatrics.* 2005;116(6):1413-26.
81. Brent NB, Redd B, Dworetz A, D'Amico F, Greenberg JJ. Breast-feeding in a low-income population. Program to increase incidence and duration. *Arch Pediatr Adolesc Med.* 1995;149(7):798-803.
82. Rosen IM, Krueger MV, Carney LM, Graham JA. Prenatal breastfeeding education and breastfeeding outcomes. *MCN Am J Matern Child Nurs.* 2008;33(5):315-9.
83. Chung M, Ip S, Yu W, Raman G, Trikalinos T, DeVine D, Lau J. Interventions in Primary Care to Promote Breastfeeding: A Systematic Review. Prepared for the Agency for Healthcare Research and Quality by the Tufts-New England Medical Center Evidence-based Practice Center. Report No.09-05126-EF-1. Rockville (MD): Agency for Healthcare Research and Quality, 2008.
84. Memmott MM, Bonuck KA. Mother's reactions to a skills-based breastfeeding promotion intervention. *Matern Child Nutr.* 2006;2(1):40-50.
85. Nielsen SB, Reilly JJ, Fewtrell MS, Eaton S, Grinham J, Wells JC. Adequacy of milk intake during exclusive breastfeeding: a longitudinal study. *Pediatrics.* 2011;128(4):e907-14.
86. Food and Agriculture Organisation. Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. Rome, 17–24 October 2001. Rome: United Nations University, World Health Organization & Food And Agriculture Organization of the United Nations; 2004.

87. Reilly JJ, Ashworth S, Wells JC. Metabolisable energy consumption in the exclusively breast-fed infant aged 3-6 months from the developed world: a systematic review. *Br J Nutr.* 2005;94(1):56-63.
88. Lucas A, Ewing G, Roberts SB, Coward WA. How much energy does the breast fed infant consume and expend? *Br Med J (Clin Res Ed).* 1987;295(6590):75-7.
89. da Costa TH, Haisma H, Wells JC, Mander AP, Whitehead RG, Bluck LJ. How much human milk do infants consume? Data from 12 countries using a standardized stable isotope methodology. *J Nutr.* 2010;140(12):2227-32.
90. Coward WA, Sawyer MB, Whitehead RG, Prentice AM, Evans J. New method for measuring milk intakes in breast-fed babies. *Lancet.* 1979;2(8132):13-4.
91. Coward WA, Cole TJ, Sawyer MB, Prentice AM. Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. *Hum Nutr Clin Nutr.* 1982;36(2):141-8.
92. Yang Z, Lönnerdal B, Adu-Afarwuah S, Brown KH, Chaparro CM, Cohen RJ, Domellöf M, Hernell O, Lartey A, Dewey KG. Prevalence and predictors of iron deficiency in fully breastfed infants at 6 mo of age: comparison of data from 6 studies. *Am J Clin Nutr.* 2009;89(5):1433-40.
93. Dewey KG, Cohen RJ, Rivera LL, Brown KH. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. *Am J Clin Nutr.* 1998;67(5):878-84.
94. Wharf SG, Fox TE, Fairweather-Tait SJ, Cook JD. Factors affecting iron stores in infants 4-18 months of age. *Eur J Clin Nutr.* 1997;51(8):504-9.
95. Lartey A, Manu A, Brown KH, Dewey KG. Predictors of Micronutrient Status among Six- to Twelve-Month-Old-Breast-Fed Ghanaian Infants. *J Nutr.* 2000, 130(2):199-207.
96. Male C, Persson LA, Freeman V, Guerra A, van't Hof MA, Haschke F; Euro-Growth Iron Study Group. Prevalence of iron deficiency in 12-mo-old infants from 11 European areas and influence of dietary factors on iron status (Euro-Growth study). *Acta Paediatr.* 2001;90(5):492-8.
97. Thorsdottir I, Gunnarsson BS, Atladottir H, Michaelsen KF, Palsson G. Iron status at 12 months of age – effects of body size, growth and diet in a population with high birth weight. *Eur J Clin Nutr.* 2003;57(4):505-513.
98. Dewey KG, Chaparro CM. Session 4: Mineral metabolism and body composition Iron status of breast-fed infants. *Proc Nutr Soc.* 2007;66(3):412-22.
99. Berglund S, Westrup B, Domellöf M. Iron supplements reduce the risk of iron deficiency anemia in marginally low birth weight infants. *Pediatrics.* 2010;126(4):874-83.

100. Hutton EK, Hassan ES. Late vs. early clamping of the umbilical cord in full-term neonates: systematic review and meta-analysis of controlled trial. *JAMA*. 2007;297(11):1241-52.
101. Ceriani Cernadas JM, Carroli G, Pellegrini L, Ferreira M, Ricci C, Casas O, Lardizabal J, Morasso Mdel C. The effect of early and delayed umbilical cord clamping on ferritin levels in term infants at six months of life: a randomized, controlled trial. *Arc Argent Pediatr*. 2010;108(3):201-8.
102. Chaparro CM, Neufeld LM, Tena Alavez G, Eguia-Líz Cedillo R, Dewey KG. Effect of timing of umbilical cord clamping on iron status in Mexican infants: a randomised controlled trial. *Lancet*. 2006;367(9527):1997-2004.
103. Domellöf M, Lönnerdal B, Dewey KG, Cohen RJ, Rivera LL, Hernell O. Sex differences in iron status during infancy. *Pediatrics*. 2002;110(3):545-52.
104. Chaparro CM. Setting the stage for child health and development: prevention of iron deficiency in early infancy. *J Nutr*. 2008;138(12):2529-33.
105. Wieringa FT, Berger J, Dijkhuizen MA, Hidayat A, Ninh NX, Utomo B, Wasantwisut E, Winichagoon P. Sex differences in prevalence of anaemia and iron deficiency in infancy in a large multi-country trial in South-East Asia. *Br J Nutr*. 2007;98(5):1070-6.
106. Mercer JS, Erickson-Owens DA. Rethinking placental transfusion and cord clamping issues. *J Perinat Neonatal Nurs*. 2012;26(3):202-17.
107. Ziegler EE, Nelson SE & Jeter JM. Iron status of breastfed infants is improved equally by medicinal iron and iron-fortified cereal. *Am J Clin Nutr*. 2009;90(1):76-87.
108. Makrides M, Leeson R, Gibson RA, Simmer K. A randomized controlled clinical trial of increased dietary iron in breast-fed infants. *J Pediatr*. 1998;133(4):559-62.
109. Domellöf M, Cohen RJ, Dewey KG, Hernell O, Rivera LL, Lönnerdal B. Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age. *J Pediatr*. 2001;138(5):679-87.
110. Lind T, Lönnerdal B, Persson L-A, Stenlund H, Tennefors K, Hernell O. Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and zinc: a randomized intervention in infants from 6 to 12 months of age. *Am J Clin Nutr*. 2003;78(1):168-75.
111. Hay G, Sandstad B, Whitelaw A, Borch-Iohnsen B. Iron status in a group of Norwegian children aged 6-24 months. *Acta Paediatr*. 2004;93(5):592-8.

112. Lozoff B, De Andraca I, Castillo M, Smith JB, Walter T, Pino P. Behavioral and developmental effects of preventing iron deficiency anemia in healthy full-term infants. *Pediatrics*. 2003;112(4):846–54.
113. Michaelsen KF, Milman N, Samuelson G. A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors. *Acta Paediatr*. 1995;84(9):1035–44.
114. Nagpal J, Sachdev H.P.S, Singh T, Mallika V. A Randomized Placebo-controlled Trial of Iron Supplementation in Breastfed Young Infants Initiated in Complementary Feeding: Effect on Haematological Status. *J Health Popul Nutr*. 2004;22(2):203-11.
115. Lozoff B, Jimenez B, Wolf AW. Long-term developmental outcome of infants with iron deficiency. *N Engl J Med*. 1991;325(10):687-94.
116. Shafir T, Angulo-Barroso R, Jing Y, Angelilli ML, Jacobson SW, Lozoff B. Iron deficiency and infant motor development. *Early Hum Dev*. 2008;84(7):479-85.
117. Ziegler EE, Nelson SE, Jeter JM. Iron supplementation of breastfed infants. *Nutr Rev*. 2011;69(1):S71-7.
118. Saarinen UM, Siimes MA, Dallman PR. Iron absorption in infants: high bioavailability of breast milk iron as indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin. *J Pediatr*. 1977;91(1):36–9.
119. Hicks PD, Zavaleta N, Chen Z, Abrams SA, Lönnerdal B. Iron deficiency, but not anemia, upregulates iron absorption in breast-fed Peruvian infants. *J Nutr*. 2006;136(9):2435–8.
120. Chantry CJ, Howard CR, Auinger P. Full breastfeeding duration and risk for iron deficiency in U.S. infants. *Breastfeed Med*. 2007;2(2):63-73.
121. Dube K, Schwartz J, Mueller MJ, Kalhoff H, Kersting M. Iron intake and iron status in breastfed infants during the first year of life. *Clin Nutr*. 2010;29(6):773-8.
122. Barker DJ. Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition*. 1997;13(9):807-13.
123. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ*. 2000;320(7240):967-71.
124. Karaolis-Danckert N, Buyken AE, Kulig M, Kroke A, Forster J, Kamin W, Schuster A, Hornberg C, Keil T, Bergmann RL, Wahn U, Lau S. How pre- and postnatal risk factors modify the effect of rapid weight gain in infancy and early childhood on subsequent fat mass development: results from the Multicenter Allergy Study 90. *Am J Clin Nutr*. 2008;87(5):1356-64.

125. Eriksson JG, Forsén T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *BMJ*. 1999;318(7181):427-31.
126. Nobili V, Alisi A, Panera N, Agostoni C. Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review. *Pediatr Endocrinol Rev*. 2008;6(2):241-7.
127. Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens*. 2000;18(7):815-31.
128. Chrestani MA, Santos IS, Horta BL, Dumith SC, de Oliveira Dode MA. Associated Factors for Accelerated Growth in Childhood: A Systematic Review. *Matern Child Health J*. 2012. [Epub ahead of print]
129. Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Gruszfeld D, Dobrzanska A, Sengier A, Langhendries JP, Rolland Cachera MF, Grote V; European Childhood Obesity Trial Study Group. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. *Am J Clin Nutr*. 2009;89(6):1836-45.
130. Escribano J, Luque V, Ferre N, Mendez-Riera G, Koletzko B, Grote V, Demmelmair H, Bluck L, Wright A, Closa-Monasterolo R; European Childhood Obesity Trial Study Group. Effect of protein intake and weight gain velocity on body fat mass at 6 months of age: the EU Childhood Obesity Programme. *Int J Obes* (Lond). 2012;36(4):548-53.
131. Gunnarsdottir I, Thorsdottir I. Relationship between growth and feeding in infancy and body mass index at the age of 6 years. *Int J Obes Relat Metab Disord*. 2003;27(12):1523-7.
132. Mahrshahi S, Battistutta D, Magarey A, Daniels LA. Determinants of rapid weight gain during infancy: baseline results from the NOURISH randomised controlled trial. *BMC Pediatr*. 2011;7(11):99.
133. Nielsen GA, Thomsen BL, Michaelsen KF. Influence of breastfeeding and complementary food on growth between 5 and 10 months. *Acta Paediatr*. 1998;87(9):911-7.
134. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. Breast feeding and obesity: cross sectional study. *BMJ*. 1999;319(7203):147-50.
135. Gopinath B, Subramanian I, Flood VM, Baur LA, Pfund N, Burlutsky G, Mitchell P. Relationship between breast-feeding and adiposity in infants and pre-school children. *Public Health Nutr*. 2012; 28:1-6.

136. Griffiths LJ, Smeeth L, Hawkins SS, Cole TJ, Dezateux C. Effects of infant feeding practice on weight gain from birth to 3 years. *Arch Dis Child*. 2009;94(8):577-82.
137. Aarts C, Kylberg E, Hofvander Y, Gebre-Medhin M. Growth under privileged conditions of healthy Swedish infants exclusively breastfed from birth to 4-6 months: a longitudinal prospective study based on daily records of feeding. *Acta Paediatr*. 2003;92(2):145-51.
138. Gunnarsdottir I, Schack-Nielsen L, Michaelsen KF, Sørensen TI, Thorsdottir I; NordNet Study Group. Infant weight gain, duration of exclusive breast-feeding and childhood BMI - two similar follow-up cohorts. *Public Health Nutr*. 2010;13(2):201-7.
139. Sloan S, Gildea A, Stewart M, Sneddon H, Iwaniec D. Early weaning is related to weight and rate of weight gain in infancy. *Child Care Health Dev*. 2008;34(1):59-64.
140. Socha P, Grote V, Gruszfeld D, Janas R, Demmelmair H, Closa-Monasterolo R, Subías JE, Scaglioni S, Verduci E, Dain E, Langhendries JP, Perrin E, Koletzko B; European Childhood Obesity Trial Study Group. Milk protein intake, the metabolic-endocrine response, and growth in infancy: data from a randomized clinical trial. *Am J Clin Nutr*. 2011;94(6):1776S-1784S.
141. Hörnell A, Lagström H, Lande B, Thorsdottir I. Breastfeeding, introduction of other foods and effects on health: A systematic literature review for the 5th Nordic Nutrition Recommendations. *Food and Nutrition Research*. [In press].
142. Owen CG, Martin RM, Whincup PH, Davey-Smith G, Gillman MW, Cook DG. The effect of breastfeeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. *Am J Clin Nutr*. 2005;82(6):1298-307.
143. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. *Pediatrics*. 2005;115(5):1367-77.
144. Kramer MS, Guo T, Platt RW, Shapiro S, Collet JP, Chalmers B, Hodnett E, Sevkovskaya Z, Dzikovich I, Vanilovich I; PROBIT Study Group. Breastfeeding and infant growth: biology or bias? *Pediatrics*. 2002;110(2 Pt 1):343-7.
145. Grummer-Strawn LM, Reinold C, Krebs NF; Centers for Disease Control and Prevention (CDC). Use of World Health Organization and CDC growth charts for children aged 0-59 months in the United States. *MMWR Recomm Rep*. 2010;59(RR-9):1-15.
146. WHO Multicentre Growth Reference Study Group. Enrolment and baseline characteristics in the WHO Multicentre Growth Reference Study. *Acta Paediatr Suppl*. 2006;450:7-15.

147. de Onis M, Garza C, Onyango AW, Borghi E. Comparison of the WHO child growth standards and the CDC 2000 growth charts. *J Nutr.* 2007;137(1):144-8.
148. de Onis M, Garza C, Onyango AW, Rolland-Cachera MF; le Comité de nutrition de la Société française de pédiatrie. WHO growth standards for infants and young children. *Arch Pediatr.* 2009;16(1):47-53.
149. WHO Multicentre Growth Reference Study Group. Assessment of differences in linear growth among populations in the WHO Multicentre Growth Reference Study. *Acta Paediatr Suppl.* 2006;450:56-65.
150. Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, Igumnov S, Fombonne E, Bogdanovich N, Ducruet T, Collet JP, Chalmers B, Hodnett E, Davidovsky S, Skugarevsky O, Trofimovich O, Kozlova L, Shapiro S; Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group. Breastfeeding and child cognitive development: new evidence from a large randomized trial. *Arch Gen Psychiatry.* 2008;65(5):578-84.
151. Jedrychowski W, Perera F, Jankowski J, Butscher M, Mroz E, Flak E, Kaim I, Lisowska-Miszczuk I, Skarupa A, Sowa A. Effect of exclusive breastfeeding on the development of children's cognitive function in the Krakow prospective birth cohort study. *Eur J Pediatr.* 2012;171(1):151-8.
152. Rao MR, Hediger ML, Levine RJ, Naficy AB, Vik T. Effect of breastfeeding on cognitive development of infants born small for gestational age. *Acta Paediatr.* 2002;91(3):267-74.
153. Oddy WH, Kendall GE, Li J, Jacoby P, Robinson M, de Klerk NH, Silburn SR, Zubrick SR, Landau LI, Stanley FJ. The long-term effects of breastfeeding on child and adolescent mental health: a pregnancy cohort study followed for 14 years. *J Pediatr.* 2010;156(4):568-74.
154. Halpern R, Giugliani ER, Victora CG, Barros FC, Horta BL. Risk factors for suspicion of developmental delays at 12 months of age. *J Pediatr (Rio J).* 2000;76(6):421-8.
155. Paterson J, Iusitini L, Gao W. Child developmental assessment at two-years of age: data from the Pacific Islands Families Study. *Pac Health Dialog.* 2011;17(2):51-63.
156. Eickmann SH, de Lira PI, Lima Mde C, Coutinho SB, Teixeira Mde L, Ashworth A. Breast feeding and mental and motor development at 12 months in a low-income population in northeast Brazil. *Paediatr Perinat Epidemiol.* 2007;21(2):129-37.
157. Kramer MS, Fombonne E, Igumnov S, Vanilovich I, Matush L, Mironova E, Bogdanovich N, Tremblay RE, Chalmers B, Zhang X, Platt RW; Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group. Effects of prolonged and exclusive breastfeeding on child behavior and maternal adjustment: evidence from a large, randomized trial. *Pediatrics.* 2008;121(3):e435-40.



158. Whitehouse AJ, Robinson M, Li J, Oddy WH. Duration of breast feeding and language ability in middle childhood. *Paediatr Perinat Epidemiol.* 2011;25(1):44-52.
159. Quinn PJ, O'Callaghan M, Williams GM, Najman JM, Andersen MJ, Bor W. The effect of breastfeeding on child development at 5 years: a cohort study. *J Paediatr Child Health.* 2001;37(5):465-9.
160. Tozzi AE, Bisiacchi P, Tarantino V, Chiarotti F, D'Elia L, De Mei B, Romano M, Gesualdo F, Salmaso S. Effect of duration of breastfeeding on neuropsychological development at 10 to 12 years of age in a cohort of healthy children. *Dev Med Child Neurol.* 2012;54(9):843-8.
161. Dee DL, Li R, Lee LC, Grummer-Strawn LM. Associations between breastfeeding practices and young children's language and motor skill development. *Pediatrics* 2007;119(1):S92-8.
162. Vestergaard M, Obel C, Henriksen TB, Sørensen HT, Skajaa E, Ostergaard J. Duration of breastfeeding and developmental milestones during the latter half of infancy. *Acta Paediatr.* 1999;88(12):1327-32.
163. Sacker A, Quigley MA, Kelly YJ. Breastfeeding and developmental delay: findings from the millennium cohort study. *Pediatrics.* 2006;118(3):e682-9.
164. Dewey KG, Cohen RJ, Brown KH, Rivera LL. Effects of exclusive breastfeeding for four versus six months on maternal nutritional status and infant motor development: results of two randomized trials in Honduras. *J Nutr.* 2001;131(2):262-7.
165. Zhou SJ, Baghurst P, Gibson RA, Makrides M. Home environment, not duration of breast-feeding, predicts intelligence quotient of children at four years. *Nutrition.* 2007;23(3):236-41.
166. Quigley MA, Hockley C, Carson C, Kelly Y, Renfrew MJ, Sacker A. Breastfeeding is associated with improved child cognitive development: a population-based cohort study. *J Pediatr.* 2012;160(1):25-32.
167. Fewtrell MS, Morley R, Abbott RA, Singhal A, Isaacs EB, Stephenson T, MacFadyen U, Lucas A. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. *Pediatrics.* 2002;110(1 Pt 1):73-82.
168. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr.* 1999;70(4):525-35.
169. Slykerman RF, Thompson JM, Becroft DM, Robinson E, Pryor JE, Clark PM, Wild CJ, Mitchell EA. Breastfeeding and intelligence of preschool children. *Acta Paediatr.* 2005;94(7):832-7.
170. Anderson LM, Shinn C, Fullilove MT, Scrimshaw SC, Fielding JE, Normand J, Carande-Kulis VG; Task Force on Community Preventive Services. The

- effectiveness of early childhood development programs. A systematic review. *Am J Prev Med.* 2003;24(3):32-46.
171. American Academy of Pediatrics, Council on Children With Disabilities; Section on Developmental Behavioral Pediatrics; Bright Futures Steering Committee; Medical Home Initiatives for Children With Special Needs Project Advisory Committee. Identifying infants and young children with developmental disorders in the medical home: an algorithm for developmental surveillance and screening. *Pediatrics.* 2006;118(1):405-20.
  172. Glascoe FP. The Brigance Infant and Toddler Screen: standardization and validation. *J Dev Behav Pediatr.* 2002;23(3):145-50.
  173. Glascoe FP, Squires J. Issues with the new developmental screening and surveillance policy statement. *Pediatrics.* 2007;119(4):861-2;discussion 862-3.
  174. Gunnlaugsson G, Saemundsson E. Að finna frávik í þroska og hegðun fimm ára barna (Finding deviation in development and behaviour of 5 year old children). Í *Ungir Íslendingar í ljósi vísindanna*, p.237-245. 2005. Reykjavík: Representative for children and the University of Iceland. 2005.
  175. Glascoe FP. Parents' evaluation of developmental status: how well do parents' concerns identify children with behavioral and emotional problems? *Clin Pediatr (Phila).* 2003;42(2):133-8.
  176. Glascoe FP. Evidence-based approach to developmental and behavioural surveillance using parents' concerns. *Child Care Health Dev.* 2000;26(2):137-49.
  177. Sices L, Stancin T, Kirchner L, Bauchner H. PEDS and ASQ developmental screening tests may not identify the same children. *Pediatrics.* 2009;124(4):e640-7.
  178. Limbos MM, Joyce DP. Comparison of the ASQ and PEDS in screening for developmental delay in children presenting for primary care. *J Dev Behav Pediatr.* 2011;32(7):499-511.
  179. Corvalán C, Kain J, Weisstaub G, Uauy R. Impact of growth patterns and early diet on obesity and cardiovascular risk factors in young children from developing countries. *Proc Nutr Soc.* 2009;68(3):327-37.
  180. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. *BMJ.* 2005;331(7522):929.
  181. Weng SF, Redsell SA, Swift JA, Yang M, Glazebrook CP. Systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy. *Arch Dis Child.* 2012;97(12):1019-26.
  182. Monteiro PO, Victora CG. Rapid growth in infancy and childhood and obesity in later life--a systematic review. *Obes Rev.* 2005;6(2):143-54.

183. Butte NF. Impact of infant feeding practices on childhood obesity. *J Nutr.* 2009;139(2):412S-6S.
184. De Kroon ML, Renders CM, Buskermolen MP, Van Wouwe JP, van Buuren S, Hirasing RA. The Terneuzen Birth Cohort. Longer exclusive breastfeeding duration is associated with leaner body mass and a healthier diet in young adulthood. *BMC Pediatr.* 2011;11:33.
185. Chivers P, Hands B, Parker H, Bulsara M, Beilin LJ, Kendall GE, Oddy WH. Body mass index, adiposity rebound and early feeding in a longitudinal cohort (Raine Study). *Int J Obes (Lond).* 2010;34(7):1169-76.
186. Schack-Nielsen L, Sørensen Tia, Mortensen EL, Michaelsen KF. Late introduction of complementary feeding, rather than duration of breastfeeding, may protect against adult overweight. *Am J Clin Nutr.* 2010;91(3):619-27.
187. Hunsberger M, Lanfer A, Reeske A, Veidebaum T, Russo P, Hadjigeorgiou C, Moreno LA, Molnar D, De Henauw S, Lissner L, Eiben G. Infant feeding practices and prevalence of obesity in eight European countries - the IDEFICS study. *Public Health Nutr.* 2013;16(2):219-27.
188. Huus K, Ludvigsson JF, Enskär K, Ludvigsson J. Exclusive breastfeeding of Swedish children and its possible influence on the development of obesity: a prospective cohort study. *BMC Pediatr.* 2008;8:42.
189. Novaes JF, Lamounier JA, Colosimo EA, Franceschini SC, Priore SE. Breastfeeding and obesity in Brazilian children. *Eur J Public Health.* 2012;22(3):383-9.
190. Durmuş B, van Rossem L, Duijts L, Arends LR, Raat H, Moll HA, Hofman A, Steegers EA, Jaddoe VW. Breast-feeding and growth in children until the age of 3 years: the Generation R Study. *Br J Nutr.* 2011;105(11):1704-11.
191. Balaban G, Motta ME, Silva GA. Early weaning and other potential risk factors for overweight among preschool children. *Clinics (Sao Paulo).* 2010;65(2):181-7.
192. Al-Qaoud N, Prakash P. 'Can breastfeeding and its duration determine the overweight status of Kuwaiti children at the age of 3-6 years?' *Eur J Clin Nutr.* 2009;63(8):1041-3.
193. Hediger ML, Overpeck MD, Kuczmarski RJ & Ruan WJ. Association between infant breastfeeding and overweight in young children. *JAMA.* 2001;285(19):2453-60.
194. Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet JP, Martin RM, Davey Smith G, Gillman MW, Chalmers B, Hodnett E, Shapiro S; PROBIT Study Group. Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity, and blood pressure at age 6.5 y: evidence from a large randomized trial. *Am J Clin Nutr.* 2007;86(6):1717-21.

195. Taveras EM, Scanlon KS, Birch L, Rifas-Shiman SL, Rich-Edwards JW, Gillman MW. Association of Breastfeeding With Maternal Control of Infant Feeding at Age 1 Year. *Pediatrics*. 2004;114(5):e577-83.
196. Taveras EM, Rifas-Shiman SL, Scanlon KS, Grummer-Strawn LM, Sherry B, Gillman MW. To What Extent Is the Protective Effect of Breastfeeding on Future Overweight Explained by Decreased Maternal Feeding Restriction? *Pediatrics*. 2006;118(6):2341-8.
197. WHO. Indicators for assessing breastfeeding practices. Report of an informal meeting. Division of Child and Adolescent Health. WHO/CDD/SER/91.14.ed. Geneva: World Health Organization, 1991.
198. Haisma H, Coward WA, Albernaz E, Visser GH, Wells JC, Wright A, et al. Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants. *Eur J Clin Nutr*. 2003;57(12):1633-42.
199. Orr-Ewing AK, Heywood PF, Coward WA. Longitudinal measurements of breast milk output by a 2H<sub>2</sub>O tracer technique in rural Papua New Guinean women. *Hum Nutr Clin Nutr*. 1986;40(6):451-67.
200. Davies PS, Wells JC. Calculation of total body water in infancy. *Eur J Clin Nutr*. 1994;48(7):490-5.
201. Racette SB, Schoeller DA, Luke AH, Shay K, Hnilicka J, Kushner RF. Relative dilution spaces of 2H- and 18O-labeled water in humans. *Am J Physiol*. 1994;267(4 Pt 1):E585-90.
202. Steingrimsdottir L, Þorgeirsdottir H, Olafsdottir AS. The Icelandic National Nutrition Survey 2002. Reykjavík: Public Health Institute of Iceland; 2003.
203. Fuchs GJ, Farris RP, DeWier M, Hutchinson SW, Warrier R, Doucet H, Suskind RM. Iron status and intake of older infants fed formula vs cow milk with cereal. *Am J Clin Nutr*. 1993;58(3):343-8.
204. Glascoe FP. Using parents' concerns to detect and address developmental and behavioral problems. *J Soc Pediatr Nurs*. 1999;4(1):24-35.
205. Glascoe FP. Detecting and addressing developmental and behavioral problems in primary care. *Pediatr Nurs*. 2000;26(3):251-7.
206. Rydz D, Shevell MI, Majnemer A, Oskoui M. Developmental screening. *J Child Neurol*. 2005;20(1):4-21.
207. Brothers KB, Glascoe FP, Robertshaw NS. PEDS: developmental milestones--an accurate brief tool for surveillance and screening. *Clin Pediatr (Phila)*. 2008;47(3):271-9.

208. Glascoe FP. Technical Report for the BRIGANCE SCREENS. 2008. Reykjavik: Educational Testing Institute and Directorate of Health.
209. Glascoe FP. Screening for developmental and behavioral problems. *Ment Retard Dev Disabil Res Rev.* 2005;11(3):173-9.
210. Glascoe FP. Are overreferrals on developmental screening tests really a problem? *Arch Pediatr Adolesc Med.* 2001;155(1):54-9.
211. Handbook for infant care in Iceland – Guidelines for healthcare of 0-5 year-olds. Directorate of Health, 2010. Available at <http://www.landlaeknir.is/servlet/file/store93/item2830/version6/4138.pdf>. Accessed October 31<sup>st</sup>, 2012.
212. WHO Anthro for personal computers, version 3.1, 2010: Software for assessing growth and development of the world's children. Geneva: WHO, 2010. Available at <http://www.who.int/childgrowth/software/en/>. Accessed November 1<sup>st</sup>, 2012.
213. World Health Organization. Training course on child growth assessment. Geneva, Switzerland: WHO; 2008. Available at [http://www.who.int/childgrowth/training/module\\_c\\_interpreting\\_indicators.pdf](http://www.who.int/childgrowth/training/module_c_interpreting_indicators.pdf). Accessed October 22<sup>nd</sup>, 2012.
214. Thorisdottir AV, Thorsdottir I, Palsson GI. Nutrition and Iron Status of 1-Year Olds following a Revision in Infant Dietary Recommendations. *Anemia.* 2011;986303. Epub 2011 Jul 18.
215. Dewey KG, Cohen RJ, Rivera LL, Brown KH. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. *Am J Clin Nutr* 1998;67:878-84.
216. Johannsson E, Arngrimsson SA, Thorsdottir I, Sveinsson T. Tracking of overweight from early childhood to adolescence in cohorts born 1988 and 1994: overweight in a high birth weight population. *Int J Obes (Lond)* 2006;30:1265–71.
217. Schluter PJ, Carter S, Percival T. Exclusive and any breast-feeding rates of Pacific infants in Auckland: data from the Pacific Islands Families First Two Years of Life Study. *Public Health Nutr* 2006;9:692–9.
218. Li R, Fein SB, Chen J, Grummer-Strawn LM. Why mothers stop breastfeeding: mothers' self-reported reasons for stopping during the first year. *Pediatrics* 2008;122(suppl 2):S69–76.
219. Singer JI, Buchino JJ, Chabali R. Selected laboratory in pediatric emergency care. *Emerg Med Clin North Am.* 1986;4:377-96.
220. Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG, MacPhail P, Schmidt U, Tal A, Mayet F. Ascorbic acid prevents the dose-dependent

inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr.* 1991;53:537-41.

221. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. *Am J Clin Nutr.* 2006;84(6):1261-76.
222. Islam MM, Khatun M, Peerson JM, Ahmed T, Mollah MA, Dewey KG, Brown KH. Effects of energy density and feeding frequency of complementary foods on total daily energy intakes and consumption of breast milk by healthy breastfed Bangladeshi children. *Am J Clin Nutr.* 2008;88(1):84-94.
223. World Health Organization: Infant and young child feeding: Model Chapter for textbooks for medical students and allied health professionals. Geneva, WHO; 2009.
224. Fisk CM, Crozier SR, Inskip HM et al. Breastfeeding and reported morbidity during infancy: findings from the Southampton Women's Survey. *Matern Child Nutr.* 2011; 7(1):61-70.
225. Gubbels JS, Thijs C, Stafleu A et. al. Association of breast-feeding and feeding on demand with child weight status up to 4 years. *Int J Pediatr Obes.* 2011;6(2-2):515-22.
226. Kitsantas P and Gaffney KF. Risk profiles for overweight/obesity among preschoolers. *Early Hum Dev.* 2010;86(9):563-8.
227. Jiang M, Foster EM. Duration of Breastfeeding and Childhood Obesity: A Generalized Propensity Score Approach. *Health Serv Res.* 2012; [Epub ahead of print].

## **Papers I – V**

# Paper I



# Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: differences in breast-milk intake by stable-isotope probe<sup>1-3</sup>

Jonathan CK Wells, Olof H Jonsdottir, Patricia L Hibberd, Mary S Fewtrell, Inga Thorsdottir, Simon Eaton, Alan Lucas, Geir Gunnlaugsson, and Ronald E Kleinman

## ABSTRACT

**Background:** The WHO recommends exclusive breastfeeding (EBF) for 6 mo after birth. However, the time at which breast milk ceases to provide adequate energy and nutrition, requiring the introduction of complementary foods, remains unclear. Most studies that investigated this issue were observational and potentially confounded by variability in social circumstances or infant growth.

**Objective:** We hypothesized that EBF infants would consume more breast milk at age 6 mo than infants receiving breast milk and complementary foods.

**Design:** We measured anthropometric outcomes, body composition, and breast-milk intake at age 6 mo in infants who were randomly assigned at age 4 mo either to 6-mo EBF or to the introduction of complementary foods with continued breastfeeding. We recruited 119 infants from health centers in Reykjavik and neighboring municipalities in Iceland. In 100 infants who completed the protocol (50/group), breast-milk intake was measured by using stable isotopes, and complementary food intakes were weighed over 3 d in the complementary feeding (CF) group.

**Results:** Breast-milk intake was 83 g/d (95% CI: 19, 148 g/d) greater in EBF (mean  $\pm$  SD: 901  $\pm$  158 g/d) than in CF (818  $\pm$  166 g/d) infants and was equivalent to 56 kcal/d; CF infants obtained 63  $\pm$  52 kcal/d from complementary foods. Estimated total energy intakes were similar (EBF: 560  $\pm$  98 kcal/d; CF: 571  $\pm$  97 kcal/d). Secondary outcomes (anthropometric outcomes, body composition) did not differ significantly between groups.

**Conclusions:** On a group basis, EBF to age 6 mo did not compromise infant growth or body composition, and energy intake at age 6 mo was comparable to that in CF infants whose energy intake was not constrained by maternal breast-milk output. *Am J Clin Nutr* 2012;96:73–9.

## INTRODUCTION

Since 2001 the WHO has recommended exclusive breastfeeding (EBF) for the first 6 mo of life in all populations (1). These recommendations were officially adopted by the governments of some but not all industrialized populations, including the United Kingdom in 2003 (2, 3). In the developing world, where the benefits of EBF to reduce diarrheal disease are paramount, the recommendations have been widely adopted.

Despite widespread official endorsement, the scientific basis of the recommendations has been questioned, attributable in part to limited evidence on when EBF ceases to provide adequate energy

and nutrition, and hence when complementary foods should be introduced alongside breast milk. According to published data on breast milk (intake, energy content) and infant energy requirements, average intakes would fail to meet energy requirements in the average 6-mo-old infant (4), although this approach relied on various assumptions about breast-milk energy content. However, insufficient relevant evidence is available, particularly in EBF infants. The appropriate time for introducing complementary foods therefore remains uncertain.

A major limitation is the scarcity of data from randomized trials (5, 6). Mothers who breastfeed their infants at all differ from those who do not breastfeed, and those who undertake 6 mo EBF differ from those who breastfeed for shorter periods (7–11). Fewer than 1% of UK mothers were practicing EBF up to age 6 mo in 2005 (12), and those who did were highly educated and well financially supported during this period (13). Reported phenotypic differences between EBF and non-EBF infants might arise either from different nutritional intakes or from differences in parental or socioeconomic circumstances. Two randomized trials of 4 compared with 6 mo of EBF were conducted in Honduras, where low birth weight is common (14, 15). In each trial, breast-milk intake was greater in the 6-mo EBF group, but anthropometric outcomes did not differ between groups. Equivalent data from populations with larger body size during infancy are lacking.

The effect of introducing complementary foods is also controversial, as such foods, which are variable, may add to or replace

<sup>1</sup> From the Childhood Nutrition Research Centre (JCKW, MSF, and AL) and the Department of Surgery (SE), UCL Institute of Child Health, London, United Kingdom; the Unit for Nutrition Research, Landspítali National University Hospital of Iceland, and Faculty of Food Science and Human Nutrition, University of Iceland, Reykjavik, Iceland (OHJ and IT); the Division of Global Health (PLH) and the Department of Pediatrics (REK), Massachusetts General Hospital for Children, Harvard Medical School, Boston, MA; and the Directorate of Health and Reykjavik University, Reykjavik, Iceland (GG).

<sup>2</sup> Supported by Mead Johnson and the Eimskip Fund of the University of Iceland.

<sup>3</sup> Address correspondence and requests for reprints to JCK Wells, Childhood Nutrition Research Centre, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom. E-mail: jonathan.wells@ucl.ac.uk.

Received November 8, 2011. Accepted for publication April 2, 2012.

First published online May 16, 2012; doi: 10.3945/ajcn.111.030403.

breast milk in the infant diet (16). In observational studies, infants receiving complementary foods earlier in infancy may also have systematically different initial growth patterns or other characteristics than those receiving complementary foods later in infancy (11). We conducted a randomized trial to evaluate the effect of timing of introduction of complementary foods on infants whose mothers practiced EBF for the first 4 mo. The study was conducted in Iceland, where EBF rates at age 6 mo were 12% (17). Mothers who agreed to participate were randomly assigned either to continue EBF for 6 mo (EBF group) or to introduce complementary foods alongside breast milk at age 4 mo [complementary feeding (CF) group]. The primary hypothesis was that breast-milk intake at 6 mo would be greater in EBF than in CF infants. Secondary hypotheses were that complementary food intake would differ between groups, whereas anthropometric outcomes and body composition would not.

## SUBJECTS AND METHODS

Recruitment for the study was undertaken from November 2007 to November 2009, with ethical permission granted by the National Bioethical Committee in Iceland and the Massachusetts General Hospital Institutional Review Board. Data collection was approved by the Data Protection Authority in Iceland. Mothers provided written informed consent to participate.

### Recruitment

Mothers were recruited from 7 health centers in the Reykjavik Capital Area and neighboring municipalities in Iceland. Mothers were informed about the study when their infant was ~2 mo old and were given study handouts if the infant was still exclusively breastfeeding at 3 mo of age. If the infant was still exclusively breastfeeding at 4 mo and the mother was interested in the study, they were invited to participate. Mothers and infants attended a screening visit and, after providing informed consent, were evaluated for the following eligibility criteria:

Infants: singleton birth, gestational age  $\geq 37$  wk, healthy (ie, absence of congenital abnormalities or chronic health issues likely to affect growth, development, or iron status), and exclusively breastfeeding at the time of assessment

Mothers: willing to be randomly assigned either to continue to breastfeed exclusively until infant age of 6 mo or to introduce complementary foods alongside breast milk at infant age of 4 mo

The operational definition of EBF was breastfeeding with no additional liquid or solid foods other than vitamins and medications (18, 19), although up to a maximum of 10 feedings of formula or water during the first 6 mo were allowed because of practicalities of EBF. Newborn infants are at times given formula or sugar water at birth and may be given extra fluids after returning home. Eligible infant-mother pairs were randomly assigned to the 2 feeding groups. Vitamin D supplementation was provided to both groups. Mothers in both groups received counseling from nurses who were lactation specialists (International Board Certified Lactation Consultants). Specifically, EBF mothers were advised to exclusively breastfeed for 6 mo and CF mothers were advised to introduce complementary foods within 7 d of being randomly assigned. During the 2-mo study

period, mothers were encouraged to contact the research staff at the Unit for Nutrition Research (Reykjavik, Iceland) and a specialist at the health care centers if they had any questions or concerns.

### Background information

Baseline data on maternal age and education (categorized here dichotomously as with/without university education), parity, and mode of delivery were collected on eligible mother-infant pairs.

### Anthropometric measurements

Data on body weight and length and head circumference were abstracted from the infants' charts at birth, and the infants were measured again at 4 and 6 mo by using Seca 757 scales and a Seca model 207 infantometer (Vogel & Halke) and flexible non-stretching tape. BMI was calculated as weight divided by length squared. All data were converted to *z*-scores by using current WHO reference data (20). Maternal weight was also measured at 6 mo (Seca 761 scales; Vogel & Halke).

### Dietary assessment

A 3-d weighed food record was obtained when the infant was aged  $157 \pm 7$  d to estimate the intake of complementary foods in the CF group. Parents were advised to keep records for 3 consecutive days and to weigh and record all complementary foods given over this period by using electronic scales accurate to 1 g (Philips model HR 2385). Data on diet were collected during the 4–6-mo study period to determine the date of introduction of the first new food item in both groups. Detailed weighed measurements were not made in the EBF group, but diary records were obtained in all subjects in case complementary foods were initiated in the EBF group before measuring breast-milk intake. Energy intakes were calculated from complementary foods by using nutrient calculation software (ICEFOOD, version 2002; Public Health Institute of Iceland) (21), with added information about infant foods.

### Isotope measurements

Breast-milk intake was determined by the deuterium dose-to-the-mother method (22, 23). Briefly, each mother received orally ~10 g deuterium oxide ( $^2\text{H}_2\text{O}$ ) diluted in ~50 g drinking water. The dose consumed was determined accurately to 0.01 g. Pre-dose urine samples were obtained from mothers and infants. Additional urine samples were collected from the mothers on days 1, 4, and 14 and from the infants on days 1, 3, 4, 13, and 14. A second isotope dose (0.05 g deuterium/kg body weight) was given orally to the infants on day 14 to calculate total body water; further infant urine samples were collected 5 h after the dose and on day 16.

Infant urine samples were obtained by placing an absorbent cotton pad in the diaper, which was checked every 30 min for wetness. The time of urination was taken as the midpoint between the last time it was dry and the time it was wet. Urine was transferred to 2-mL cryogenic tubes and stored at  $-20^\circ\text{C}$ . Urine and dose samples were transported frozen to the United Kingdom for isotope-ratio mass spectrometry (Delta XP; Thermo

Fisher Scientific). Samples were analyzed in duplicate (precision 1.4 delta units) by using the equilibration method.

### Modeling

Total body water was calculated by using the back-extrapolation method in both mothers and infants (24). Dilution spaces were assumed to overestimate body water by a factor of 1.044 (25). Breast-milk intake was calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover in the mothers and infants, and the transfer of milk from mother to infant, on the basis of equations and assumptions described previously (22, 23). A “solver” function in Excel (Microsoft) was used to minimize the sum of squares of differences between observed and fitted values for maternal and infant data combined.

### Statistical analyses

The sample size calculations were based on the primary hypothesis that EBF infants would have higher breast-milk intakes than CF infants. Fifty mother-infant pairs per group allowed us to detect differences of  $\geq 75$  g/d with 80% power and 5% significance, which is equivalent to  $\sim 0.6$  SD (23). Recruitment continued until 100 mother-infant pairs completed the study. Independent *t* tests and chi-square tests were used to compare results between groups. Regression analysis, with a dummy variable for feeding group, was used to test group differences in breast milk with adjustment for confounding variables. Exploratory analyses were used to identify factors associated with EBF mothers introducing any non-breast-milk foods during the period

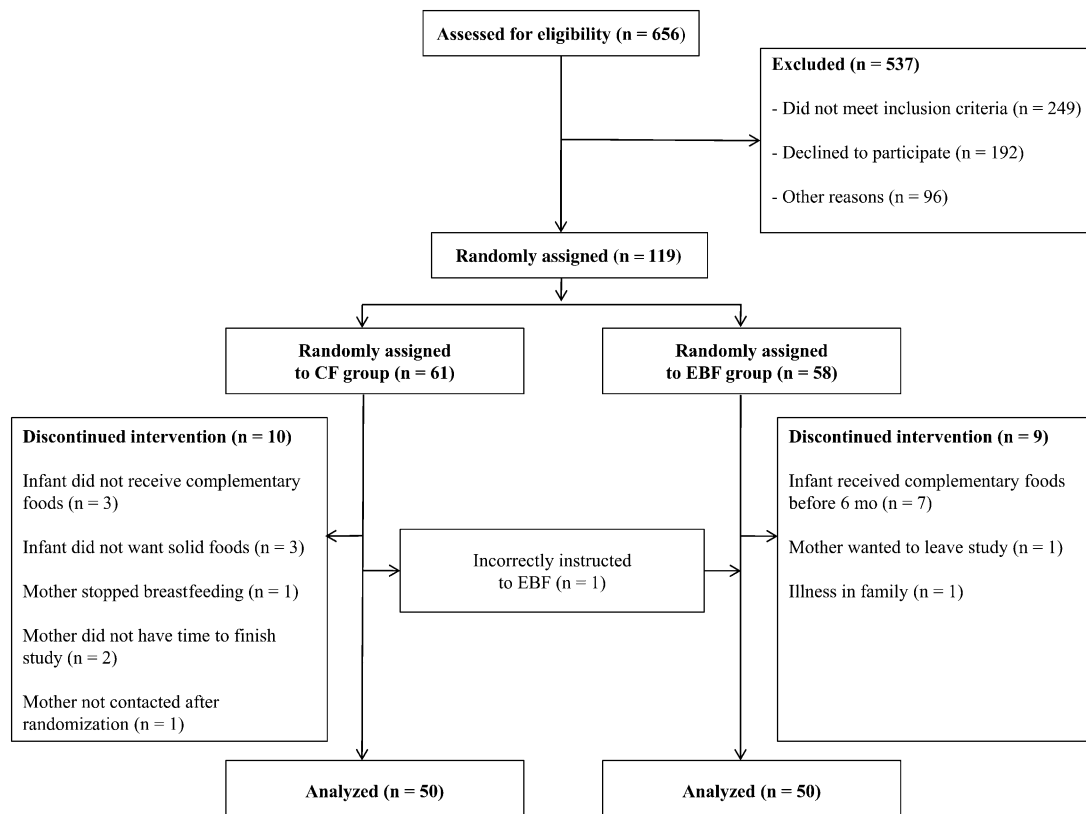
of breast-milk measurement. The index of potential harm assessed was growth status.

### Randomization

With the use of specially designed software ([www.randomization.com](http://www.randomization.com)), permuted blocks of 2 and 4 with the sequence presented in random order were used to generate assignments, which were accessed by using a password-protected Web-based application after eligibility criteria were confirmed. Assignments were generated by one person who was not involved in any other aspect of the study. After randomization, one mother who was randomly assigned to the CF group was incorrectly instructed to the EBF group. We performed the primary analyses with this mother included in the EBF group ( $n = 50$  EBF, 50 CF) but have reported outcomes for the baseline analyses with this subject in the CF group ( $n = 49$  EBF, 51 CF). Nurses who collected data on complementary food intakes and anthropometric outcomes were not blinded to participant group status, but all mass spectrometric analyses and isotopic modeling were blinded.

### RESULTS

A total of 119 mother-infant pairs were recruited, of whom 100 completed the trial protocol (**Figure 1**). Among the 10 CF mothers who did not complete the study, either their infants were not given, or did not accept, complementary foods or the mothers stopped breastfeeding or did not complete the study. Seven EBF infants were given complementary foods after 4 mo, whereas 2 EBF mothers did not complete the study. The primary



**FIGURE 1.** Profile of the recruitment process. CF, complementary feeding; EBF, exclusive breastfeeding.

outcome (milk intake) was not measured in the infants whose mothers did not comply with the protocol.

Data on maternal and infant characteristics at birth or at the time of randomization are provided in **Table 1**. There were few differences between completers and noncompleters; however, noncompleting infants were 0.9 cm (95% CI: -0.1, 1.9 cm) longer at 4 mo, which was significant when expressed in *z*-scores (0.42; 95% CI: 0.01, 0.82; *P* = 0.04). Noncompleters were also more likely to be primiparous (dropouts: 10 of 19, 52.6%; completers: 27 of 100, 27%; *P* = 0.027). Within the EBF group, noncompleting infants did not differ in 4-mo anthropometric outcomes compared with completing infants. Within the EBF group, noncompleting mothers were younger than completing mothers (mean  $\pm$  SD: 25.0  $\pm$  3.4 compared with 30.9  $\pm$  5.1 y; NS) and were significantly more likely to be primiparous (*n* = 6 of 8 compared with *n* = 10 of 50, *P* < 0.001).

The mother-infant pairs in the 2 groups who completed the trial are also compared in Table 1. There were no differences in maternal characteristics or in infant anthropometric outcomes, except that a smaller proportion of the EBF infants were delivered vaginally (46% compared with 36%, *P* = 0.03), and there was a borderline-significant trend for fewer primiparous mothers in the EBF group (*P* = 0.057).

Infant anthropometric outcomes and breast-milk intake at age 6 mo are shown in **Table 2**. Although one mother was instructed to join the wrong group, all mothers in the final analysis successfully followed the isotope protocol for breast-milk intake. Relative to WHO reference values, the sample had significantly greater anthropometric outcome *z*-scores in both groups from birth to age 6 mo. However, there were no significant differences between groups in anthropometric outcomes (**Figure 2**) or in lean or fat mass by deuterium dilution.

Mean ( $\pm$ SD) breast-milk intake in the EBF group was 901  $\pm$  158 g/d, which was higher than the WHO reference values of 854  $\pm$  24 g/d ( $\Delta$  = 47 g/d; 95% CI: 2, 92 g/d; *P* = 0.04). The CF group consumed 818  $\pm$  166 g breast milk/d, which was not significantly different from the WHO reference values ( $\Delta$  = -36; 95% CI: -83, 11 g/d). The EBF group therefore consumed 83 g/d (95% CI: 19, 148 g/d) more breast milk than did the CF group (*P* = 0.012). Regression analysis showed that, holding constant for primiparity and mode of delivery, the EBF group still consumed significantly more breast milk ( $\Delta$  = 83 g/d; 95% CI: 18, 154 g/d). None of the findings for growth, body composition, or breast-milk intake ( $\Delta$  = 76; 95% CI: 11, 141 g/d) changed if the analysis was conducted for the 2 groups as originally randomized (ie, 51 CF compared with 49 EBF infants).

The average daily intake of complementary foods among CF infants was 92.2 g and included 43% water given as a drink or as part of foods prepared by the parents, such as porridge. The remaining foods included 17% infant formula, 16% fruit purée, 12% fruit/vegetables, 9% infant cereal (dry mass), and small amounts of other foods. Mean ( $\pm$ SD) daily energy intake was 63.4  $\pm$  52.3 kcal, which is equivalent to 265.4  $\pm$  219 kJ, of which 49% was attributable to infant cereals, 12% to fruit/vegetables, 18% to infant formula, 13% to fruit purée, 3% to oils or butter, 1% to infant vitamin or cod liver oil, and 4% to other foods.

During the 2-wk period of isotope measurement, a small proportion of mothers in the EBF group gave their infants either water (*n* = 4; 8% of the group), sugar water (*n* = 1; 2%), formula milk (*n* = 3; 6%), or rice porridge (*n* = 2; 4%). In total, 6 mothers (12%) gave their infants some energy-containing fluids or foods other than breast milk. In most cases, however, the amounts were

**TABLE 1**

Baseline characteristics of subjects who completed the study compared with dropouts and of the 2 groups of mothers and infants<sup>1</sup>

	Completing ( <i>n</i> = 100)	Noncompleting ( <i>n</i> = 19)	<i>P</i>	CF group ( <i>n</i> = 51)	EBF group ( <i>n</i> = 49)	<i>P</i>
<b>Maternal data</b>						
University education ( <i>n</i> )	54	11	0.76 <sup>2</sup>	31	24	0.23 <sup>2</sup>
Primiparous ( <i>n</i> )	27	10	0.027 <sup>2</sup>	18	9	0.057 <sup>2</sup>
Multiparous ( <i>n</i> )	73	9		33	40	
Vaginal delivery ( <i>n</i> )	83	89	0.48 <sup>2</sup>	46	36	0.02 <sup>2</sup>
Weight (kg)	—	—	—	73.0 $\pm$ 15.5	71.3 $\pm$ 12.4	0.5
Age (y)	30.2 $\pm$ 4.8 <sup>3</sup>	28.1 $\pm$ 6.9	0.22	29.7 $\pm$ 4.4	30.7 $\pm$ 5.1	0.2
<b>Infant data</b>						
Male ( <i>n</i> )	46	9	0.9 <sup>2</sup>	25	21	0.5 <sup>2</sup>
Age at enrollment (d)	123.0 $\pm$ 3.3	122.0 $\pm$ 3.9	0.77	123.0 $\pm$ 3.4	123.0 $\pm$ 3.2	0.95
Gestational age (d)	280.9 $\pm$ 8.3	278.6 $\pm$ 11.5	0.29	279.6 $\pm$ 8.8	277.9 $\pm$ 8.1	0.3
Birth weight (kg)	3.71 $\pm$ 0.48	3.67 $\pm$ 0.37	0.75	3.70 $\pm$ 0.44	3.71 $\pm$ 0.52	0.9
Birth weight <i>z</i> -score <sup>4</sup>	0.83 $\pm$ 0.94	0.77 $\pm$ 0.65	0.77	0.82 <sup>5</sup> $\pm$ 0.89	0.83 <sup>5</sup> $\pm$ 1.00	0.9
Weight at age 4 mo (kg)	7.0 $\pm$ 0.9	7.0 $\pm$ 0.6	1.00	7.05 $\pm$ 1.00	6.99 $\pm$ 0.81	0.9
Weight <i>z</i> -score <sup>4</sup>	0.33 $\pm$ 0.95	0.37 $\pm$ 0.57	0.89	0.34 <sup>5</sup> $\pm$ 1.05	0.33 <sup>5</sup> $\pm$ 0.83	0.9
Length at age 4 mo (cm)	64.4 $\pm$ 2.1	65.1 $\pm$ 1.8	0.13	64.3 $\pm$ 2.3	64.5 $\pm$ 1.9	0.7
Length <i>z</i> -score <sup>4</sup>	0.65 $\pm$ 0.86	1.07 $\pm$ 0.59	0.04	0.58 <sup>5</sup> $\pm$ 0.91	0.72 <sup>5</sup> $\pm$ 0.80	0.4

<sup>1</sup> All comparisons were made by independent *t* test except where indicated otherwise. CF, complementary feeding; EBF, exclusive breastfeeding.

<sup>2</sup> Chi-square test.

<sup>3</sup> Mean  $\pm$  SD (all such values).

<sup>4</sup> Calculated by using WHO reference data (18).

<sup>5</sup> Significantly different from WHO mean values, *P* < 0.05.



**TABLE 2**Age, size, body composition, and breast-milk intake of the infants at age 6 mo<sup>1</sup>

	CF group (n = 50)	EBF group (n = 50)	Difference between groups <sup>2</sup>	P
Age (d)	186.7 ± 7.1 <sup>3</sup>	187.3 ± 6.6	0.6 (−3.3, 2.2) <sup>4</sup>	0.6
Weight (kg)	7.96 ± 1.06	8.01 ± 1.04	0.05 (−0.47, 0.37)	0.8
Weight z-score	0.28 ± 1.08	0.36 <sup>5</sup> ± 0.99	−0.08 (−0.49, 0.33)	0.7
Length z-score	0.60 <sup>5</sup> ± 0.92	0.77 <sup>5</sup> ± 0.84	0.17 (−0.52, 0.18)	0.3
BMI z-score	−0.08 ± 1.14	−0.10 ± 1.04	−0.03 (−0.41, 0.46)	0.9
Head circumference z-score	0.94 <sup>5</sup> ± 0.77	1.02 <sup>5</sup> ± 0.89	0.07 (−0.26, 0.40)	0.6
Lean mass (kg) <sup>6,7</sup>	5.13 ± 0.92	4.96 ± 1.18	−0.17 (−0.28, 0.62)	0.4
Fat mass (kg) <sup>6,7</sup>	2.71 ± 0.96	3.04 ± 1.12	0.33 (−0.77, 0.11)	0.14
Breast-milk intake <sup>6</sup> (g/d)	818 ± 166	901 ± 158	83 (19, 148)	0.012

<sup>1</sup> CF, complementary feeding; EBF, exclusive breastfeeding.<sup>2</sup> Differences between groups tested by independent *t* test<sup>3</sup> Mean ± SD (all such values).<sup>4</sup> Mean; 95% CI in parentheses (all such values).<sup>5</sup> Significantly different from WHO mean values by paired *t* test, *P* < 0.05.<sup>6</sup> Determined by using isotope measurements.<sup>7</sup> *n* = 43 and 46 for CF and EBF groups, respectively.

very small, occurred on one day only, and contained very little energy. Because the isotope method estimates deuterium transfer from the mother, these extra fluids do not compromise the accuracy of breast-milk intake values. Only 2 mothers gave their infants any significant quantity of energy other than breast milk during the isotope study period: one fed her infant 200 mL of infant formula on one day only, whereas one mother provided 4–5 spoons of rice porridge almost daily. Breast-milk intake of the 6 infants who received any extra calories was marginally (26 g/d; 95% CI: −166, 113 g/d) higher than that in the remaining 44 EBF infants (mean ± SD intakes: 924 ± 192 compared with 898 ± 155 g/d), suggesting they were hungrier, but the difference was not significant. If these 6 infants were excluded, the remaining 44 EBF infants still consumed significantly more breast milk than did the 50 CF group infants ( $\Delta$  = 80 g/d; 95% CI: 13, 146 g/d; *P* = 0.018).

## DISCUSSION

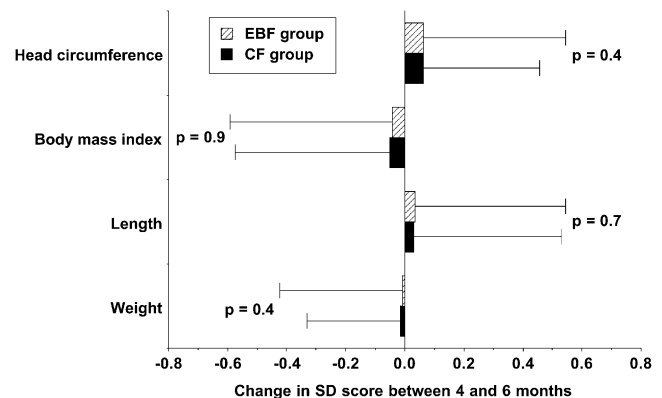
Our trial, which, to our knowledge, is the first of its kind in a population with average growth rates consistent with current WHO reference data throughout infancy, showed that infants of mothers randomly assigned to 6 mo of EBF showed satisfactory growth and had average breast-milk intakes slightly above WHO reference values (26). Infants randomly assigned to complementary foods at age 4 mo consumed significantly less breast milk at age 6 mo. Our approach allowed experimental disentangling of the effect of introducing complementary foods on breast-milk intake from the effects of baseline maternal or infant factors that may affect both infant diet and growth. Our findings were similar to those of 2 trials in Honduras, a population with a high frequency of low-birth-weight infants, where breast-milk intake at age 6 mo was greater in EBF than in CF infants, although growth rates were similar (14, 15).

The comparison of intakes of breast milk and complementary foods between groups of infants, whose behavior or social circumstances may drive the pattern of weaning, has long been a challenge. Measurement of breast-milk intake is also challenging, because intakes are “invisible” and cannot be readily

studied using approaches common in other age groups. Techniques adapted for infants, such as test-weighing, may intrude on the normal behavior of mothers and/or infants and hence bias the results (27). Despite the enormous emphasis placed on the value of breastfeeding for short- and long-term health, objective data on breast-milk intakes remain sparse, although a review of objective isotope data from 12 countries was recently published (28). A third challenge is that rates of EBF at age 6 mo are typically <15% in industrialized populations [Canada, 13.8%; United States, 11.3%; Sweden, 10.1%; Norway, 7% (29–32)] and only ~1% in the United Kingdom (12).

We addressed these challenges in a randomized trial in Iceland by using an established isotope method to obtain objective data on breast-milk intake. In a national cohort in 1995–1997, rates of EBF in Iceland were 88% at 1 mo, 69% at 3 mo, and 46% at 4 mo (33), whereas in a 2005–2007 cohort, rates were higher (34).

Previous data on our research question are sparse. In a review of observational data from 8 developed country populations, the mean (±SD) breast-milk intake in 93 EBF infants at age 6 mo was 854 ± 118 g/d, although individual population averages ranged from 800 ± 120 to 925 ± 112 g/d (26). There is concern



**FIGURE 2.** Changes in anthropometric z-scores between ages 4 and 6 mo in EBF and CF groups. The average changes did not differ between groups (all *P* values were >0.4). Error bars represent SD. CF, complementary feeding; EBF, exclusive breastfeeding.



regarding the utility of these test-weighting values from highly selected populations as a reference. Randomized trials enable these concerns to be addressed. Data from 2 randomized trials from Honduras indicated no adverse effect of 6 mo of EBF on growth (14, 15), but whether this applies to populations with larger infant sizes requires confirmation.

The mothers in our trial showed a high degree of compliance with the protocol. The majority (88%) of completing EBF mothers reported giving no energy-containing food to their infants up to the end of the isotope measurement, and of those who provided such foods, only 2 did so in any significant quantity. The exclusion of these infants did not affect our findings. However, another 7 EBF infants were dropped from the study because they were given complementary foods; hence, the total EBF compliance rate was 77%. For CF infants, the compliance rate was 82%. Because the primary outcomes were not measured in those not complying with the full protocol, our analysis was restricted to the 100 infants with isotopic data.

We calculated energy consumption with the assumption that the metabolizable energy content of breast milk at age 6 mo is 62.1 kcal/100 g (4). Current WHO guidelines for energy requirements in breastfed infants at age 6 mo are 78 kcal · kg<sup>-1</sup> · d<sup>-1</sup> (35), which in this study would equate to 625 kcal/d for the average EBF infant in this study. The EBF infants received 560 ± 98.2 kcal/d, which is equivalent to 90.1% of the recommendation. The CF group received 508 ± 103 kcal/d from breast milk plus 63 ± 53.2 kcal/d from complementary foods, totaling 571 ± 97 kcal/d, which is equivalent to 92.7% of the recommendation. Our data therefore show an apparent inconsistency, in that the infants appear to meet WHO recommendations for breast-milk intake but not energy intake. This may be because the WHO recommendations were calculated from total energy expenditure data in predominantly, not exclusively, breastfed infants. Introducing formula milk or cow milk into the diet increases infant energy expenditure (35, 36); hence, the value of 78 kcal · kg<sup>-1</sup> · d<sup>-1</sup> may be an overestimate of actual energy requirements in EBF infants. It is also possible that the energy content of breast milk in our population is >62.1 kcal/100g, and this merits further research.

The similar estimated energy intakes of the 2 groups indicate that complementary foods acted primarily to replace breast milk. Given the similarity between groups in body weight throughout the trial and in body composition and estimated energy intake at age 6 mo, we assume that infant appetite was the key factor driving energy intake.

There was no indication of energy inadequacy in the EBF infants. Anthropometric outcome *z*-scores were consistently above zero; although this partly reflects the infants' large size at birth, which is typical in the Icelandic population (37), *z*-scores at ages 4 and 6 mo in the 2 groups were very similar, as was lean mass at age 6 mo, giving no indication of faltering in the EBF group. However, each group also showed variability, and we were unable to determine whether those infants within each group who grew less between birth and 6 mo had insufficient breast-milk intakes earlier in infancy.

We have highlighted the fact that previously published evidence on breast-milk intakes did not support the proposition that the average infant could satisfy his or her energy requirements at age 6 mo from breast milk alone (4, 38). This suggestion was not intended to undermine breastfeeding but rather to promote further research into this important issue. Mothers themselves commonly

cite concern that their breast milk has become insufficient as a source of nutrition for their infant as a reason for introducing formula-milk or complementary foods (39, 40); hence, evidence on the breast-milk intakes of EBF infants will help encourage mothers to breastfeed. In the present study, we have shown that the large majority (77%) of a sample of mothers practicing EBF at 4 mo successfully continued EBF to 6 mo, with their infants showing adequate growth. Another longitudinal study, in Glasgow, showed that EBF mothers recruited from breastfeeding support groups increased breast-milk output between 4 and 6 mo (41). Thus, both of these studies rebut our challenge and suggest that EBF successfully meets energy requirements, but further work will be required to determine whether all mothers in this and other populations could achieve this outcome.

The strengths of our study include its randomized design and the use of an objective method for measuring breast-milk intake. Conducting the study in Iceland represents both a strength, because EBF is sufficiently common there for a randomized trial to be feasible, and a limitation, because the cold environment and high birth weight are not typical of most European populations, which reduces generalizability. The 2 feeding groups differed at baseline in the mode of delivery and maternal parity; however, these differences were relatively minor and did not account for our main findings regarding breast-milk or complementary food intakes. We were not able to test whether those who dropped out of the study were those with lower breast milk intakes—ie, potentially a self-selected group. Finally, our study was designed to evaluate growth and energy intake and not other issues such as development of dietary preferences, mineral status, or effects on health such as diarrhea and allergy.

We are grateful to those who participated and to colleagues for the following contributions. The recruitment, data collection on breastfeeding, and provision of support for mothers were undertaken by the nurses: Alma Maria Rognvaldsdóttir, Audur Egilsdóttir, Dagny Gudmundsdóttir, Sesselja Gudmundsdóttir, Elin Sigurbjörnsdóttir, Gudrun Gudmundsdóttir, Ingibjörg Eiríksdóttir, Jóna Margret Jónsdóttir, and Kristín Vigfusdóttir. Dietician Margret Thora Jónsdóttir and PhD student Tinna Eysteinsdóttir are also gratefully acknowledged for their contributions to data collection. The study was also supported by the Primary Health Care organizations in the Reykjavík Capital Area, Akranes, and Sudurnes, and by the directors of the participating health centers. Anna Fiorino prepared the randomization method using Jerry Dallal's Tufts-based software, developed the Web-based data entry system, and synthesized the study database. James Grinham performed mass spectrometric analyses at UCL Institute of Child Health. The protocol can be obtained from Ronald E Kleinman.

The responsibilities of the authors were as follows:—GG, AL, MSF, REK, PLH, JCKW, and IT: designed the study; OHJ: was responsible for the nutritional and isotope data collection; SE: was responsible for the mass spectrometric analyses; and JCKW: undertook the isotope calculations and, after extensive discussions among the study team, conducted the statistical analysis and wrote the first draft of the manuscript. All authors critically revised the manuscript and had full access to all of the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis. The study sponsors had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the manuscript for publication. None of the authors declared a conflict of interest.

## REFERENCES

1. World Health Organization. Infant and young child nutrition. 55th World Health Assembly. Geneva, Switzerland: World Health Organization, 2002.

2. Scientific Advisory Committee on Nutrition (SACN). Paper for discussion: introduction of solid foods, agenda item: 3. Subgroup on Maternal and Child Nutrition. London, United Kingdom: The Stationery Office, 2003.
3. American Academy of Pediatrics. Breastfeeding and the use of human milk. *Pediatrics* 2005;115:496–506.
4. Reilly JJ, Ashworth S, Wells JC. Metabolisable energy consumption in the exclusively breast-fed infant aged 3–6 months from the developed world: a systematic review. *Br J Nutr* 2005;94:56–63.
5. Kramer MS, Kakuma R. The optimal duration of exclusive breastfeeding: a systematic review. *Adv Exp Med Biol* 2004;554:63–77.
6. Fewtrell M, Wilson DC, Booth I, Lucas A. Six months of exclusive breast feeding: how good is the evidence? *BMJ* 2011;342:c5955.
7. Motil KJ, Kertz B, Thotathuchery M. Lactational performance of adolescent mothers shows preliminary differences from that of adult women. *J Adolesc Health* 1997;20:442–9.
8. Fewtrell MS, Lucas A, Morgan JB. Factors associated with weaning in full term and preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2003;88:F296–301.
9. Griffiths LJ, Tate AR, Dezateux C. Do early infant feeding practices vary by maternal ethnic group? *Public Health Nutr* 2007;10:957–64.
10. Hawkins SS, Griffiths LJ, Dezateux C, Law C. The impact of maternal employment on breast-feeding duration in the UK Millennium Cohort Study. *Public Health Nutr* 2007;10:891–6.
11. Wright CM, Parkinson K, Scott J. Breast-feeding in a UK urban context: who breast-feeds, for how long and does it matter? *Public Health Nutr* 2006;9:686–91.
12. Bolling K, Grant C, Hamlyn C, Thornton A. Infant feeding survey 2005. Leeds, United Kingdom: The Information Centre, 2007.
13. Kelly YJ, Watt RG. Breast-feeding initiation and exclusive duration at 6 months by social class—results from the Millennium Cohort Study. *Public Health Nutr* 2005;8:417–21.
14. Cohen RJ, Brown KH, Canahuati J, Rivera LL, Dewey KG. Effects of age of introduction of complementary foods on infant breast milk intake, total energy intake, and growth: a randomised intervention study in Honduras. *Lancet* 1994;344:288–93.
15. Dewey KG, Cohen RJ, Brown KH, Rivera LL. Age of introduction of complementary foods and growth of term, low-birth-weight, breast-fed infants: a randomized intervention study in Honduras. *Am J Clin Nutr* 1999;69:679–86.
16. Quandt SA. The effect of beikost on the diet of breast-fed infants. *J Am Diet Assoc* 1984;84:47–51.
17. Gunnlaugsson G. Brjóstgjöf á Íslandi. Börn fædd árið 1999–2002. [Breastfeeding in Iceland. Children born 1999–2002.] Reykjavik, Iceland: Miðstöð heilsuverndar barna (Centre for Child Health Services), 2005 (in Icelandic).
18. WHO. Indicators for assessing breastfeeding practices. Report of an informal meeting. Division of Child and Adolescent Health. Geneva, Switzerland: WHO, 1991. (Publication WHO/CDD/SER/91.14.)
19. WHO, UNICEF, USAID, Academy for Educational Development, University of California Davis, International Food Policy Research Institute. Indicators for assessing infant and young child feeding practices—part I: Definitions. Conclusions of a consensus meeting held 6–8 November 2007 in Washington, DC, 2008. Geneva, Switzerland: WHO, 2008.
20. WHO. Software for assessing growth and development of the world's children, version 3.1. Geneva, Switzerland: WHO, 2010.
21. Steingrimsdottir L, Þorgeirsdottir H, Olafsdottir AS. The Icelandic National Nutrition Survey 2002. Reykjavik, Iceland: Public Health Institute of Iceland, 2003.
22. Coward WA, Cole TJ, Sawyer MB, Prentice AM. Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. *Hum Nutr Clin Nutr* 1982;36:141–8.
23. Haisma H, Coward WA, Albernaz E, Visser GH, Wells JC, Wright A, Victora CG. Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants. *Eur J Clin Nutr* 2003;57:1633–42.
24. Davies PS, Wells JC. Calculation of total body water in infancy. *Eur J Clin Nutr* 1994;48:490–5.
25. Racette SB, Schoeller DA, Luke AH, Shay K, Hnilicka J, Kushner RF. Relative dilution spaces of 2H- and 18O-labeled water in humans. *Am J Physiol* 1994;267:E585–90.
26. Butte NF, Lopez-Alarcon MG, Garza C. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. Geneva, Switzerland: WHO, 2002.
27. Lucas A, Ewing G, Roberts SB, Coward WA. How much energy does the breast fed infant consume and expend? *Br Med J (Clin Res Ed)* 1987;295:75–7.
28. da Costa TH, Haisma H, Wells JC, Mander AP, Whitehead RG, Bluck LJ. How much human milk do infants consume? Data from 12 countries using a standardized stable isotope methodology. *J Nutr* 2010;140:2227–32.
29. Al-Sahab B, Lanes A, Feldman M, Tamim H. Prevalence and predictors of 6-month exclusive breastfeeding among Canadian women: a national survey. *BMC Pediatr* 2010;10:20.
30. Lande B, Andersen LF, Baerug A, Trygg KU, Lund-Larsen K, Veierod MB, Bjørneboe GE. Infant feeding practices and associated factors in the first six months of life: the Norwegian infant nutrition survey. *Acta Paediatr* 2003;92:152–61.
31. Brekke HK, Ludvigsson JF, van Odijk J, Ludvigsson J. Breastfeeding and introduction of solid foods in Swedish infants: the All Babies in Southeast Sweden study. *Br J Nutr* 2005;94:377–82.
32. Centers for Disease Control and Prevention. Breastfeeding trends and updated national health objectives for exclusive breastfeeding—United States, birth years 2000–2004. *MMWR Morb Mortal Wkly Rep* 2007;56:760–3.
33. Atladottir H, Thorsdottir I. Energy intake and growth of infants in Iceland—a population with high frequency of breast-feeding and high birth weight. *Eur J Clin Nutr* 2000;54:695–701.
34. Thorisdottir AV, Thorsdottir I, Palsson GI. Nutrition and iron status of 1-year-olds following a revision in infant dietary recommendations. *Anemia* 2011;2011:986303.
35. Food and Agriculture Organization. Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. Rome, 17–24 October 2001. Rome, Italy: United Nations University, WHO/FAO, 2004.
36. Haisma H, Wells JC, Coward WA, Filho DD, Victora CG, Vonk RJ, Wright A, Visser GH. Complementary feeding with cow's milk alters sleeping metabolic rate in breast-fed infants. *J Nutr* 2005;135:1889–95.
37. Johannsson E, Arngrimsson SA, Thorsdottir I, Sveinsson T. Tracking of overweight from early childhood to adolescence in cohorts born 1988 and 1994: overweight in a high birth weight population. *Int J Obes (Lond)* 2006;30:1265–71.
38. Reilly JJ, Wells JC. Duration of exclusive breast-feeding: introduction of complementary feeding may be necessary before 6 months of age. *Br J Nutr* 2005;94:869–72.
39. Schluter PJ, Carter S, Percival T. Exclusive and any breast-feeding rates of Pacific infants in Auckland: data from the Pacific Islands Families First Two Years of Life Study. *Public Health Nutr* 2006;9:692–9.
40. Li R, Fein SB, Chen J, Grummer-Strawn LM. Why mothers stop breastfeeding: mothers' self-reported reasons for stopping during the first year. *Pediatrics* 2008;122(suppl 2):S69–76.
41. Nielsen SB, Reilly JJ, Fewtrell MS, Eaton S, Grinham J, Wells JC. Adequacy of milk intake during exclusive breastfeeding: a longitudinal study. *Pediatrics* 2011;128:e907–14.



# Paper II



# PEDIATRICS®

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

## **Timing of the Introduction of Complementary Foods in Infancy: A Randomized Controlled Trial**

Olof H. Jonsdottir, Inga Thorsdottir, Patricia L. Hibberd, Mary S. Fewtrell, Jonathan C. Wells, Gestur I. Palsson, Alan Lucas, Geir Gunnlaugsson and Ronald E. Kleinman  
*Pediatrics* 2012;130;1038; originally published online November 12, 2012;  
DOI: 10.1542/peds.2011-3838

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://pediatrics.aappublications.org/content/130/6/1038.full.html>

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2012 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™



# Timing of the Introduction of Complementary Foods in Infancy: A Randomized Controlled Trial



**WHAT'S KNOWN ON THIS SUBJECT:** In a previous randomized trial, infants from a low-resource country exclusively breastfed for 6 months had lower iron stores at 6 months compared with breastfeeding infants receiving solid foods. Randomized trials of exclusive breastfeeding in high-income countries are lacking.



**WHAT THIS STUDY ADDS:** In a high-income country, infants who receive complementary foods in addition to breast milk from 4 months of age had higher iron stores at 6 months compared with those exclusively breastfed for 6 months.

## abstract

**OBJECTIVE:** To increase knowledge on iron status and growth during the first 6 months of life. We hypothesized that iron status would be better in infants who received complementary foods in addition to breast milk compared with those exclusively breastfed.

**METHODS:** One hundred nineteen healthy term ( $\geq 37$  weeks) singleton infants were randomly assigned to receive either complementary foods in addition to breast milk from age 4 months (CF) or to exclusive breastfeeding for 6 months (EBF). Dietary data were collected by 3-day weighed food records, and data on iron status and growth were also collected.

**RESULTS:** One hundred infants (84%) completed the trial. Infants in the CF group had higher mean serum ferritin levels at 6 months ( $P = .02$ ), which remained significant when adjusted for baseline characteristics. No difference was seen between groups in iron deficiency anemia, iron deficiency, or iron depletion. The average daily energy intake from complementary foods of 5-month-olds in the CF group was 36.8 kJ per kg body weight. Infants in both groups grew at the same rate between 4 and 6 months of age.

**CONCLUSIONS:** In a high-income country, adding a small amount of complementary food in addition to breast milk to infants' diets from 4 months of age does not affect growth rate between 4 and 6 months, but has a small and positive effect on iron status at 6 months. The biological importance of this finding remains to be determined. *Pediatrics* 2012;130:1038–1045

**AUTHORS:** Olof H. Jonsdottir, BSc,<sup>a</sup> Inga Thorsdottir, PhD,<sup>a</sup> Patricia L. Hibberd, MD, PhD,<sup>b</sup> Mary S. Fewtrell, MD,<sup>c</sup> Jonathan C. Wells, PhD,<sup>c</sup> Gestur I. Palsson, MD,<sup>d</sup> Alan Lucas, MD,<sup>c</sup> Geir Gunnlaugsson, MD, PhD,<sup>e</sup> and Ronald E. Kleinman, MD<sup>f</sup>

<sup>a</sup>Unit for Nutrition Research, Landspítali, The National University Hospital of Iceland and Faculty of Food Science and Nutrition, University of Iceland, Reykjavik, Iceland; <sup>b</sup>Division of Global Health, Massachusetts General Hospital for Children, Harvard Medical School, Boston, Massachusetts; <sup>c</sup>Childhood Nutrition Research Centre, University College London Institute of Child Health, London, United Kingdom; <sup>d</sup>Children's Hospital, Landspítali, The National University Hospital of Iceland, and <sup>e</sup>Directorate of Health and Reykjavik University, Reykjavik, Iceland; and <sup>f</sup>Department of Pediatrics, Massachusetts General Hospital for Children, Harvard Medical School, Boston, Massachusetts

### KEY WORDS

exclusive breastfeeding, complementary feeding, iron status, growth rate, randomized trial

### ABBREVIATIONS

CF—complementary feeding in addition to breast milk from the age of 4 months

EBF—exclusive breastfeeding to the age of 6 months

Hb—hemoglobin

ID—iron deficiency

IDA—iron deficiency anemia

MCV—mean corpuscular volume

RDW—red blood cell distribution width

SF—serum ferritin

TIBC—total iron-binding capacity

WHO—World Health Organization

This study was structured by Drs Kleinman, Gunnlaugsson, Lucas, Fewtrell, and Jane Morgan (now retired) and subsequently designed in detail by them with Drs Hibberd, Thorsdottir, and Wells. Mrs Jonsdottir was responsible for the nutritional and isotope data collection, and, following extensive discussions among the study team, she conducted the statistical analysis and wrote the first draft of the manuscript, which was critically reviewed and revised by all authors. Funding was initially sought unsuccessfully from the National Institutes of Health but subsequently provided by Mead Johnson and the Eimskip Fund of the University of Iceland. All authors had full access to the trial data for interpretation. The corresponding author was responsible for the final decision to submit the report for publication.

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, preparation of the report, or the decision to submit for publication.

This trial has been registered with the ISRCTN Register (<http://isrctn.org>) (identifier ISRCTN41946519).

(Continued on last page)

In 2001, after a World Health Organization (WHO) Expert Consultation on the Optimal Duration of Exclusive Breastfeeding<sup>1</sup> and a systematic review,<sup>2</sup> WHO changed the recommended duration of exclusive breastfeeding from the first 4 to 6 months<sup>3</sup> to the first 6 months of life. The principle reason for this change was to provide optimal nutrition to young infants in low-resource countries where available water and complementary foods may be nutritionally inadequate or contaminated.<sup>4–6</sup> In high-income countries, the evidence for recommending 6 months of exclusive breastfeeding is less clear.<sup>7</sup> One systematic review of the optimal age for the introduction of complementary foods concluded that there was inadequate evidence to increase the duration of exclusive breastfeeding from 4 to 6 months of age.<sup>8</sup> Because few randomized controlled trials are available, WHO has requested such trials be done to guide policy decisions in this regard.<sup>2</sup>

In this study, we sought to examine the optimal duration of exclusive breastfeeding by conducting a masked, randomized, controlled trial comparing the iron status and growth rate of infants in Iceland randomized to 4 versus 6 months of exclusive breastfeeding. Our primary hypothesis in this analysis was that serum iron status at 6 months of age would be better in infants who received complementary foods in addition to breast milk from 4 months of age compared with those exclusively breastfed. Secondary outcomes included gains in infant weight, length, and head circumference during the study period.

## METHODS

### Study Design

This parallel group, masked, randomized controlled trial was conducted in 7 health care centers in Iceland where >46% of mothers exclusively breastfeed through 4 months of age.<sup>9,10</sup> The

study was reviewed and approved by the Data Protection Authority and National Bioethical Committee in Iceland and the Partners Health System Institutional Review Board.

Mothers of eligible infants (singleton birth, gestational length  $\geq 37$  weeks, exclusively breastfed, infant characterized as healthy; ie, absence of congenital abnormalities or chronic health issues likely to affect growth, development, or iron status) were informed about the study when their infant was  $\sim 2$  months old and given study handouts if the infant was still exclusively breastfed at age 3 months. They were contacted before the infant's 4-month birthday and, if still exclusively breastfeeding, invited to participate in the trial.

Mothers and their infants attended a screening visit and, after obtaining informed consent, were evaluated for the following additional eligibility criteria: exclusively breastfed at the time of assessment, mother willing to continue to breastfeed exclusively until 6 months or give complementary foods at 4 months based on randomization, and mother-infant pair likely able to participate for the duration of the study. Exclusive breastfeeding was defined as breastfeeding with no additional liquid or solid foods other than vitamins and medications.<sup>11</sup> The use of up to 10 feedings of formula or water during the first 6 months was allowed to avoid having to exclude infants who were otherwise exclusively breastfed.

Eligible infant-mother pairs were randomly assigned to receive complementary foods from age 4 months in addition to breast milk (CF) or to continue being exclusively breastfed to age 6 months (EBF). Vitamin D supplements were recommended in both groups. Mothers in both groups received counseling from a nurse who also was an International Board Certified Lactation Consultant. Mothers in the EBF group were advised to

breastfeed exclusively for 6 months, and mothers in the CF group were advised to give the infant complementary foods within 7 days of randomization, along with continued breastfeeding. During the 2-month study period, mothers could at all times contact the research staff at the Unit for Nutrition Research and the International Board Certified Lactation Consultant nurse at the health care centers.

### Randomization

The trial statistician provided a computer generated randomization code. Assignments were generated by using permuted blocks of 2 and 4, with the sequence presented in random order. Assignments were accessed by using a password-protected web-based application, after eligibility criteria were confirmed.

### Dietary Assessment

Mothers assigned to the CF group were asked to keep a diary to indicate the date that every new food item was added to the infant's diet from the time of enrollment into the study until the child was 6 months of age. A 3-day weighed food record was obtained when the infant reached  $\sim 5$  months and 1 week ( $\pm 7$  days) of age. Parents were asked to weigh and record every food their infant consumed over this 3-day period, using electronic scales with 1-g accuracy (Philips HR 2385, Philips, Szekesfehervar, Hungary) provided by the study team. Energy and nutrient information were calculated with the nutrient calculation software ICEFOOD (version 2002; Public Health Institute of Iceland, Reykjavik, Iceland), with added information about infant foods.<sup>12</sup>

### Anthropometric Assessment

Infants' weight, length, and head circumference were measured at birth, 6 weeks, and 3, 4, 5, and 6 months of age

at their health care center. Mothers who enrolled in the study were asked if these records could be accessed to determine their infant's growth rate for the trial period. The scales used to weigh the infants were Seca model 727 or model 757 (Vogel & Halke, Hamburg, Germany) depending on the health care center. Length was measured with the infant supine on a standard measuring board with a sliding foot plate (Seca model 207, Vogel & Halke). Head circumference was measured with nonstretchable tape. Infants were measured once at each visit by an experienced child health nurse trained specifically for the study. Weight, length, and head circumference were converted to z scores using the WHO Infant Growth Standards.<sup>13</sup>

### Blood Samples

Blood samples were obtained from the infants to determine iron status. Blood for hemoglobin (Hb), mean corpuscular volume (MCV), red blood cell distribution width (RDW), serum ferritin (SF), and total iron-binding capacity (TIBC) was obtained from the infants at age 6 months ( $\pm 7$  days) by an experienced pediatrician at the age of 6 months ( $\pm 7$  days). Analyses were done by using a Coulter Counter STKS at Landspítali National University Hospital of Iceland. The criteria for iron deficiency anemia (IDA) required that all 3 indicators met the following cutpoints: Hb  $< 105$  g/L, MCV  $< 74$  fL, and SF  $< 12$   $\mu$ g/L. The same cutpoints were used to evaluate both iron deficiency (ID; MCV  $< 74$  fL and SF  $< 12$   $\mu$ g/L) and iron depletion (SF  $< 12$   $\mu$ g/L).<sup>14–17</sup>

### Statistical Analysis

Our sample size of 50 mother-infant pairs who completed their participation in each group ( $n = 100$ ) was based on primary outcome of the study, breast-milk intake. That sample size allowed for the detection of an effect

size of 0.6 between the 2 groups with 5% significance level (2-sided comparison) and 80% power. Subjects were recruited until 100 had completed the protocol. Statistical analysis was performed by using SPSS software (version 17; SPSS Inc., Chicago, IL).

For descriptive analysis, data were presented as mean and SD when normally distributed and median and interquartile range when not normally distributed. For comparison between two groups, the independent *t* test was used when data were normally distributed and the non-parametric Mann-Whitney *U* test was used when data were not normally distributed.  $\chi^2$ -Square test or Fisher's exact test was used for dichotomous variables. Multiple regression analysis was done to evaluate the predictors of iron status indices at 6 months. If there were baseline characteristics that were significantly different between the two intervention groups, for all subjects or among those who finished the whole trial, they were adjusted for in the regression analysis.

## RESULTS

### Sample Size and Characteristics

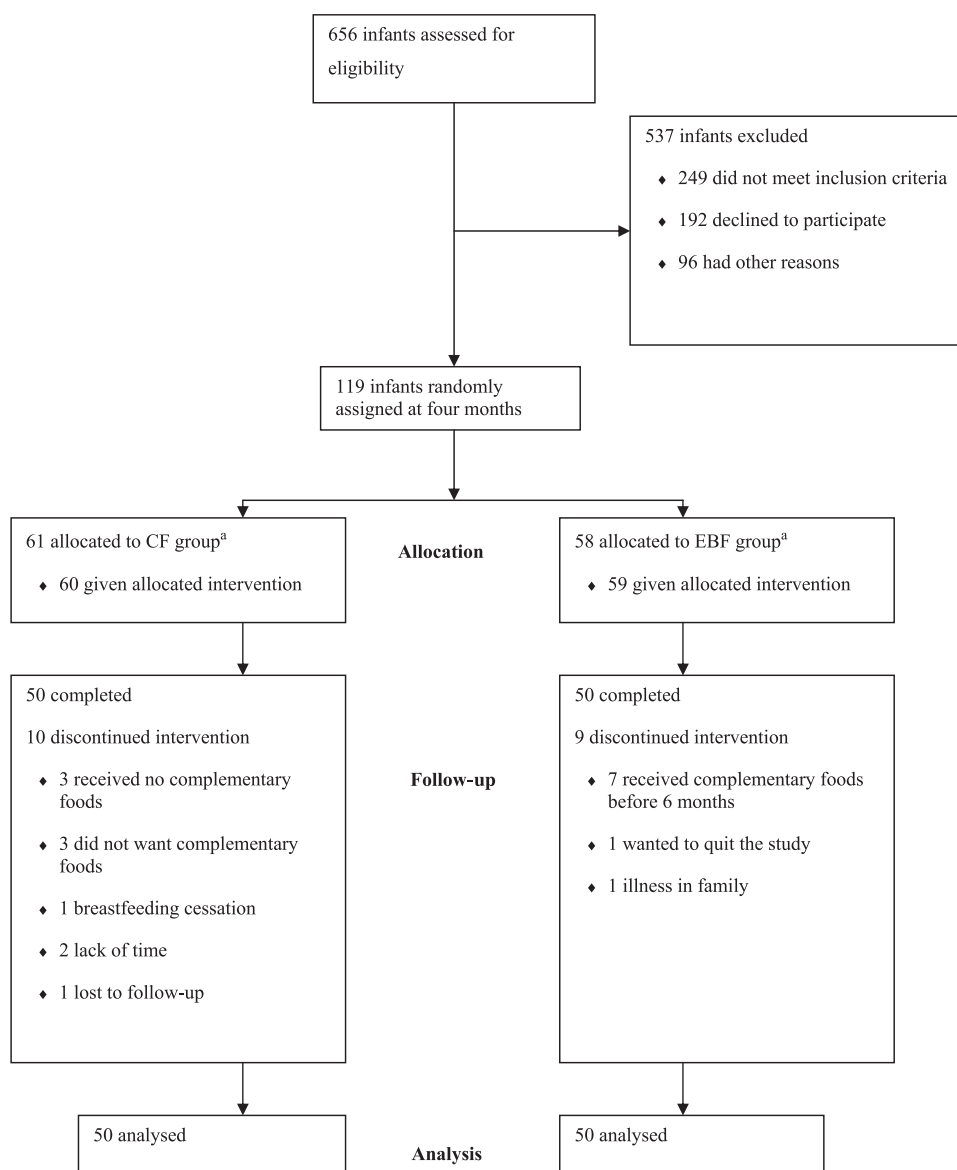
One hundred nineteen mother-infant pairs were enrolled in the study between November 2007 and November 2009 (see Fig 1). One mother-infant pair was randomized to the CF group but was incorrectly instructed to exclusively breastfeed, which she did, so in the main analyses, this infant was analyzed in the EBF group. There were 10 dropouts in the CF group and 9 in the EBF group. Mothers of infants who dropped out had significantly fewer children in the household ( $1.0 \pm 1.0$  vs  $2.0 \pm 2.0$ ;  $P = .03$ ). The infants who dropped out had higher interval increases in z score for length ( $1.07 \pm 0.59$  vs  $0.65 \pm 0.86$ ;  $P = .01$ ) and gained more length from birth to 4 months of age ( $0.16 \pm 0.90$  vs  $-.43 \pm 1.00$ ;  $P = .02$ )

compared with those who remained in the study. When categorized according to intention to treat no statistically significant differences were seen between groups in baseline characteristics in mothers or infants (see Table 1). No significant differences were seen between intervention groups in the characteristics (same as shown in Table 1) of subjects who completed the whole trial ( $n = 100$ ), except for mode of delivery, for which vaginal delivery was more common in the CF group (90% vs 73% in the EBF group,  $P = .03$ ).

### Infant Growth and Iron Status

Table 2 compares gain in weight, length, and head circumference z scores from 0 to 4, 4 to 6, and 0 to 6 months between groups. No significant differences were seen in growth rate between the groups before the intervention period (0 to 4 months) or during the intervention period (4 to 6 months) or from birth to 6 months, categorized according to infant diet during the study period.

Infants in CF group had significantly higher SF levels than those in EBF group ( $P = .02$ ) as shown in Table 2. One infant in EBF group had a urinary tract infection 2 weeks before the blood sample was taken, which might have affected the SF level at 6 months,<sup>18</sup> which was high (524  $\mu$ g/L). When this outlier was excluded from the data analysis, the infants in the CF group still had significantly higher SF level at 6 months ( $70.0 \pm 73.0$   $\mu$ g/L vs  $43.0 \pm 50.0$   $\mu$ g/L in the EBF group;  $P = .013$ ). Excluding infants ( $n = 16$ ) who had any problem that might have affected the ferritin level (infections, C-reactive protein concentration  $> 10$  mg/L<sup>19</sup> or those in group EBF who received any complementary foods before 6 months) the difference in SF level remained significant, with higher levels of SF among infants in CF group ( $67.0 \pm 61.0$   $\mu$ g/L vs  $34.0 \pm 44.5$   $\mu$ g/L in the EBF group;  $P = .003$ ).

**FIGURE 1**

Trial profile: infants who received complementary foods in addition to breast milk from 4 months compared with infants who were exclusively breastfed for 6 months. <sup>a</sup>One mother-infant pair was incorrectly instructed to group EBF and was therefore analyzed in group EBF.

Figure 2 shows box plots of SF divided into quartiles for comparison between groups; in each quartile infants who received complementary foods had higher SF levels, significant in all quartiles. There was no significant difference in Hb, MCV, TIBC, or RDW between the 2 study groups. One infant in EBF group had 2 blood samples drawn (1 at age 6 months +4 days and the other at 6 months +18 days) because some indices were inadvertently not examined on the first sample

and were therefore examined on the second sample, and values from both samples were combined into 1 report. SF was transformed because of its skewed distribution, and regression analysis was undertaken to test the impact of the intervention by group, excluding the outlier. It showed that SF differences remained significant ( $P = .035$ ) when adjusted for mode of delivery, which was significantly different between groups at baseline.

Seven infants had depleted iron stores at age 6 months, 5 of whom were in the EBF group and 2 in the CF group ( $P = .44$ ). Of those 7 infants, 6 were also ID (4 in group EBF, 2 in the CF group;  $P = .68$ ) and 2 had IDA (1 in group EBF, 1 in group CF;  $P = 1.00$ ).

### Nutrient Intake

Table 3 shows the results of weighed dietary registration for infants in the CF group and the percentage of recommended daily intake. Average daily



**TABLE 1** Baseline Characteristics of Subjects: Infants Who Received Complementary Foods in Addition to Breast Milk From 4 Months Compared With Infants Who Were Exclusively Breastfed for 6 Months,<sup>a</sup> Categorized According to Intention to Treat

	CF (n = 61)	EBF (n = 58)
<b>Infant characteristics</b>		
Gender: boys	31 (51%) <sup>b</sup>	24 (41%) <sup>b</sup>
Age at randomization (d)	122.9 (3.3)	123.1 (3.1)
Gestational length (d)	281.5 (8.4)	279.6 (9.3)
Wt for age at birth <sup>c</sup>	0.86 (0.83)	0.78 (0.96)
Birth wt (kg)	3.72 (0.43)	3.68 (0.51)
Length for age at birth <sup>c</sup>	1.07 (0.89)	1.03 (1.06)
Length at birth (cm)	51.5 (1.7)	51.4 (2.1)
Head circumference for age at birth <sup>c</sup>	1.40 (1.01) <sup>d</sup>	1.44 (1.08)
Head circumference at birth (cm)	35.9 (1.3) <sup>d</sup>	35.9 (1.4)
Wt for age at 4 mo <sup>c</sup>	0.36 (0.98)	0.32 (0.80)
Wt at 4 mo (kg)	7.07 (0.92)	6.97 (0.79)
Length for age at 4 mo <sup>c</sup>	0.68 (0.88)	0.75 (0.80)
Length at 4 mo (cm)	64.5 (2.2)	64.5 (1.9)
Head circumference for age at 4 mo <sup>c</sup>	0.93 (0.71)	0.92 (0.88)
Head circumference at 4 mo (cm)	42.3 (1.1)	42.2 (1.3)
<b>Maternal characteristics</b>		
Age at randomization (y)	29.7 (5.2)	30.1 (5.2)
Parity	2.0 (2.0) <sup>e</sup>	2.0 (2.0) <sup>e</sup>
Maternal education at university level <sup>f</sup>	36 (59%) <sup>b</sup>	29 (50%) <sup>b</sup>
Vaginal delivery	54 (89%) <sup>b</sup>	45 (78%) <sup>b</sup>

Data are mean (SD) unless otherwise indicated.

<sup>a</sup> One mother-infant pair was incorrectly instructed to follow directions for the EBF group.

<sup>b</sup> Data are presented as n (%).

<sup>c</sup> z scores calculated using WHO reference.

<sup>d</sup> One missing value, n = 60.

<sup>e</sup> Data are presented as median (interquartile range).

<sup>f</sup> Finished studies at university level.

intake of complementary foods among infants in the CF group was 92.2 ± 66.5 g. Mean energy intake from complementary foods at 5 months was 265.4 ± 219.0 kJ per day (63.4 ± 52.3 kcal/d) and 36.8 kJ/kg body weight (8.8 kcal/

**TABLE 2** Growth Rate in z Scores and Iron Status Indices in the 2 Intervention Groups, CF and EBF,<sup>a</sup> Categorized According to Infant Diet During the Study Period

Variables	CF (n = 50)	EBF (n = 50)	P value
Wt gain from 0–4 mo <sup>b</sup>	−0.54 (1.01)	−0.42 (1.04)	.51
Length gain from 0–4 mo <sup>b</sup>	−0.41 (0.85)	−0.26 (1.14)	.40
Gain in HC from 0 to 4 mo <sup>b</sup>	−0.47 (0.84) <sup>c</sup>	−0.52 (1.03)	.80
Wt gain from 4 to 6 mo	−0.02 (0.31)	−0.01 (0.42)	.90
Length gain from 4 to 6 mo	0.03 (0.50)	0.04 (0.51)	.96
Gain in HC from 4–6 mo	0.06 (0.40)	0.06 (0.48)	.99
Wt gain from 0 to 6 mo	−0.55 (1.12)	−0.46 (1.17)	.71
Length gain from 0 to 6 mo	−0.41 (0.95)	−0.37 (1.18)	.85
Gain in HC from 0 to 6 mo	−0.36 (0.87) <sup>c</sup>	−0.41 (1.01)	.80
Hb (g/L) <sup>d</sup>	113.9 (6.1)	113.7 (7.3) <sup>e</sup>	.91
MCV (fL) <sup>d</sup>	74.4 (3.3)	73.4 (2.6) <sup>e</sup>	.11
SF (μg/L) <sup>d</sup>	70.0 (73.3) <sup>f</sup>	44.0 (53.8) <sup>f</sup>	.02
TIBC (μmol/L) <sup>d</sup>	59.2 (9.2)	60.8 (10.1)	.43
RDW <sup>d</sup>	13.0 (0.7)	13.2 (0.7) <sup>e</sup>	.13

Data are mean (SD) unless otherwise indicated. HC, head circumference.

<sup>a</sup> One mother-infant pair was incorrectly instructed to follow directions for the EBF group and was therefore analyzed in that group.

<sup>b</sup> CF n = 60; EBF n = 59.

<sup>c</sup> One missing value.

<sup>d</sup> Hb, MCV, and RDW n = 98; SF n = 94; TIBC n = 95.

<sup>e</sup> Mean (SD) calculated with values from a blood sample taken 2 weeks later than 6 mo from 1 infant because some indices were inadvertently not examined on the first sample and were therefore examined on the second sample.

<sup>f</sup> Data are median (interquartile range).

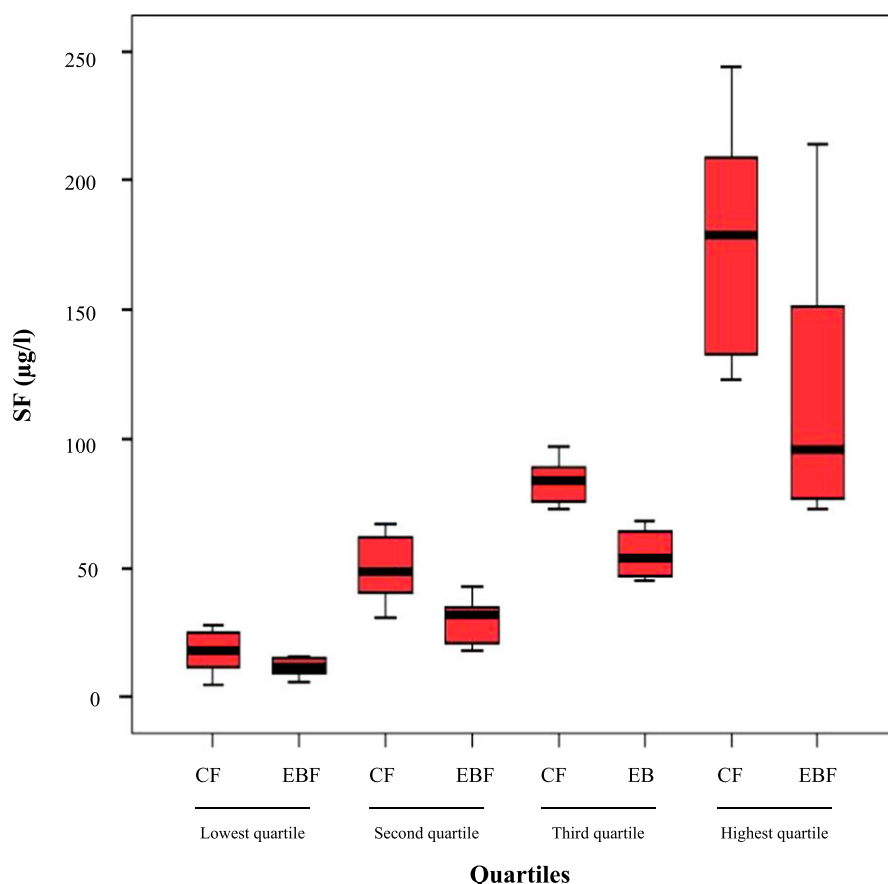
kg), which is 10% of the average daily energy requirements for infants 6 to 11 months of age.

The mean daily intake of iron from complementary foods was 0.6 mg, which is 8% of an infant's average daily iron requirements at age 6 to 11 months. The main sources of iron from the complementary foods consumed by infants in CF group were infant cereals (67%), infant formula (17%), and fruit purées (8%). Vitamin C intake from complementary foods mostly came from fruit purée (43%), infant cereals (21%), fruits and vegetables (15%), and infant formula (14%). Iron or vitamin C intake did not correlate with any iron status indices at 6 months, nor did total food or formula intake (data not shown).

## DISCUSSION

In 2001, WHO requested randomized trials of exclusive breastfeeding in both high-income and low-resource countries. To our knowledge this is the first such trial to examine the effects of exclusive breastfeeding for 4 versus 6 months on iron status and growth in a high-income country. Weight, length, and head circumference measures in these 2 groups of infants were equivalent, and no significant differences were seen between groups in growth before or during the intervention period or from birth to 6 months.

The results of the current study regarding growth are consistent with results from Kramer and Kakuma's systematic review,<sup>2</sup> which was the basis of the WHO recommendation regarding the optimal duration of exclusive breastfeeding. The review concluded that there was no reduction in growth in children exclusively breastfed for 4 versus 6 months. These findings are also consistent with our previous analysis in which infants in the CF group consumed less breast milk than those in EBF group



**FIGURE 2**

Box plot of SF divided into quartiles for comparison between groups in each quartile (1 outlier excluded). Mean and SD for each group in each quartile are presented in parentheses: lowest quartile (CF:  $17.8 \pm 8.4 \mu\text{g/L}$  vs EBF:  $11.9 \pm 3.4 \mu\text{g/L}$ ;  $n = 22$ ;  $P = .05$ ), second quartile (CF:  $49.4 \pm 12.7 \mu\text{g/L}$  vs EBF:  $29.9 \pm 8.5 \mu\text{g/L}$ ;  $n = 25$ ;  $P < .001$ ), third quartile (CF:  $83.4 \pm 7.8 \mu\text{g/L}$  vs EBF:  $55.5 \pm 8.6 \mu\text{g/L}$ ;  $n = 24$ ;  $P < .001$ ) and highest quartile (CF:  $176.6 \pm 45.2 \mu\text{g/L}$  vs EBF:  $117.4 \pm 47.5 \mu\text{g/L}$ ;  $n = 22$ ;  $P = .007$ ).

**TABLE 3** Intake of Selected Nutrients Received at 5.25 Months of Age as an Average Intake From Weighed Dietary Registration of Complementary Foods ( $n = 50$ )

	Mean (SD)	Median (IQR)	RDI (6–11 mo)	% of RDI
Energy (kJ/d)	265.4 (219.0)	178.1 (271.0)	—	—
Energy (kJ/kg)	36.8 (32.1)	22.5 (42.2)	355	10
Protein (g/d)	1.3 (1.1)	0.9 (1.3)	—	—
Protein (g/kg)	0.2 (0.2)	0.1 (0.2)	1.1	18
Iron (mg/d)	0.6 (0.5)	0.3 (0.7)	8	8
Vitamin C (mg/d)	8.8 (9.9)	3.9 (15.5)	20	44
Calcium (mg/d)	32.5 (41.7)	11.2 (46.3)	540	6
Vitamin D ( $\mu\text{g/d}$ )	4.3 (4.6)	2.5 (9.9)	10	43

All infants received breast milk in addition to the complementary foods, breast milk is not included in the table. One mother-infant pair was incorrectly instructed to group EBF and was therefore analyzed in group EBF. IQR, interquartile range; RDI, recommended daily intake. \*Recommended daily intake does not exist for energy (kJ/d) or protein (g/d).

and received their compensatory energy from complementary foods, when measured by stable isotope techniques.<sup>20</sup> Whereas infants in CF group had higher iron stores at 6 months compared with those in EBF group, both had adequate stores as

measured by SF levels and no significant differences were seen between groups in iron deficiency with or without anemia. The mode of delivery was significantly different between groups among those who finished the intervention, and this could affect the

timing of umbilical cord clamping. Delayed cord clamping can have a beneficial effect on infant iron and hematologic status through 6 months of age.<sup>21–23</sup> After adjustments for mode of delivery, the association of intervention diet with SF was still seen, suggesting that the higher iron stores were related to the complementary feeding. These findings are consistent with those of a similar study conducted in a low-resource country in which infants who were exclusively breastfed for 6 months had lower measures of iron stores at 6 months compared with infants who received solid foods in addition to breast milk ( $48.4$  vs  $67.3 \mu\text{g/L}$ ).<sup>24</sup> Other investigators have shown that infants with a higher growth rate are at

greater risk of iron deficiency,<sup>25,26</sup> thought to be accounted for by more rapidly depleted iron stores from birth with greater weight gain.<sup>27</sup> Because weight gain did not differ between groups in this study, weight gain cannot account for the differences in the observed SF levels.

In the current study, the intake of complementary foods by infants in the CF group was much lower than expected, with an average energy intake of 265.4 kJ/d and mean daily iron intake of 0.6 mg per day at age 5 months and 1 week. Average energy intake and the amount of iron that these infants received from complementary foods was small, only 59% of the mean energy intake (451 kJ/d) and 15% of the mean iron intake (4.1 mg/d) that breastfeeding infants in Honduras received from solid foods at 21 weeks of age.<sup>24</sup> In our study, we considered whether vitamin C in complementary foods can have an effect on iron absorption<sup>28</sup> but saw no relationship between vitamin C intake and measures of iron status. This is likely related to low intakes of complementary foods, and thus both iron and vitamin C, among these infants. This low complementary food intake might be explained by the manner in which the study was presented to the mothers of infants in group CF. The mothers were told to give their infant some complementary foods daily, but no amount was specified. Alternatively, these infants may have consumed more complementary foods during the course of the study than parents recorded, either overall or during the specific 3-day period when

they weighed and recorded the foods. Nevertheless, the CF infants stored significantly more iron. The biological importance of this finding remains to be seen, because both groups had values of SF considered to be within normal values, and the infants in the study were not followed beyond 6 months of age.

The dropout rate in the current study was low and similar in the 2 study groups. The length-for-age at 4 months was higher in the dropout group than among those who finished the whole intervention trial; also, the dropouts gained length faster from birth to 4 months. Thus, it is possible that some of those infants who left the study did so because of higher energy demand. Mothers who dropped out of the study had significantly fewer children in the household and were equally represented between both study groups.

A modified per protocol analysis were conducted for the mother-infant pair who was incorrectly instructed to group EBF. Whether an intent to treat or a modified per protocol analysis was conducted, the outcome remained unchanged.

Which markers should be used to detect iron deficiency is controversial. The iron status indicators and the thresholds chosen in the study are in line with studies on infant population in the neighboring countries and previous infant studies conducted in Iceland. Iron status at baseline was not possible to measure, and this is a limitation of the study.

## CONCLUSIONS

The current study showed a significant difference in SF levels between groups, and the association between the timing of the introduction of complementary foods and SF was still seen after adjustments for baseline differences. The amount of complementary foods consumed from age 4 months by infants in this study was small as measured at 5 months and did not affect the growth of these infants compared with those exclusively breastfed. In this study, conducted in an urban setting in a high-income country, the biological significance of the higher SF levels among those infants who began complementary feeding at 4 months of age remains to be determined.

## ACKNOWLEDGMENTS

We are grateful to the infants and their parents for their willingness to participate in the study. We gratefully acknowledge the nurses at the Centre for Child Health Services, Jóna Margrét Jónsdóttir, and Sesselja Guðmundsdóttir, and the nurses at the Health Care centers, Alma María Rognvaldsdóttir, Audur Egilsdóttir, Dagný Guðmundsdóttir, Elin Sigurbjörnsdóttir, Guðrún Guðmundsdóttir, Ingibjörg Eiríksdóttir, and Kristín J. Vígfúsdóttir, for recruiting mother-infant pairs and their significant assistance in data collection. We also thank dietician Margrét Thóra Jónsdóttir and PhD student Tinna Eysteinsdóttir for their assistance in collecting data and the staff in the laboratories at Landspítali National University Hospital of Iceland for blood analysis.

## REFERENCES

1. World Health Organization. *The Optimal Duration of Exclusive Breastfeeding: Report of an Expert Consultation* (WHO/NHD/01.09). Geneva, Switzerland: Department of Nutrition for Health and Development and Department of Child and Adolescent Health and Development, World Health Organization, 2001
2. Kramer MS, Kakuma R. *The Optimal Duration of Exclusive Breastfeeding: A Systematic Review* (WHO/NHD/01.08). Geneva, Switzerland: Department of Nutrition for Health and Development and Department of Child and Adolescent Health and Development, World Health Organization, 2001
3. World Health Organization. Nutrition: Information and attitudes among health personnel



- about early infant-feeding practices. *Wkly Epidemiol Rec*. 1995;70(17):117–120
4. Kramer MS, Chalmers B, Hodnett ED, et al; PROBIT Study Group (Promotion of Breast-feeding Intervention Trial). Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *JAMA*. 2001;285(4):413–420
  5. Monterrosa EC, Frongillo EA, Vásquez-Garibay EM, Romero-Velarde E, Casey LM, Willows ND. Predominant breast-feeding from birth to six months is associated with fewer gastrointestinal infections and increased risk for iron deficiency among infants. *J Nutr*. 2008; 138(8):1499–1504
  6. Kramer MS, Guo T, Platt RW, et al. Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breast-feeding. *Am J Clin Nutr*. 2003;78(2):291–295
  7. Fewtrell MS, Morgan JB, Duggan C, et al. Optimal duration of exclusive breastfeeding: what is the evidence to support current recommendations? *Am J Clin Nutr*. 2007;85(2):635S–638S
  8. Lanigan JA, Bishop JA, Kimber AC, Morgan J. Systematic review concerning the age of introduction of complementary foods to the healthy full-term infant. *Eur J Clin Nutr*. 2001;55(5):309–320
  9. Atladóttir H, Thorsdóttir I. Energy intake and growth of infants in Iceland—a population with high frequency of breast-feeding and high birth weight. *Eur J Clin Nutr*. 2000;54(9):695–701
  10. Thorisdóttir AV, Thorsdóttir I, Pálsson GI. Nutrition and iron status of 1-year olds following a revision in infant dietary recommendations. *Anemia*. 2011;2011:986303
  11. WHO. *Indicators for Assessing Breastfeeding Practices. Report of an Informal Meeting. Division of Child and Adolescent Health (WHO/CDD/SER/91.14)*. Geneva, Switzerland: World Health Organization; 1991
  12. Steingrimsdóttir L, Þorgeirsdóttir H, Ólafsdóttir AS. *The Icelandic National Nutrition Survey 2002*. Reykjavik: Public Health Institute of Iceland; 2003
  13. WHO Anthro for personal computers, version 3.1, 2010: Software for assessing growth and development of the world's children. Geneva, Switzerland: WHO, 2010. Available at: [www.who.int/childgrowth/software/en](http://www.who.int/childgrowth/software/en). Accessed October 1, 2012
  14. Thorsdóttir I, Gunnarsson BS, Atladóttir H, Michaelsen KF, Pálsson G. Iron status at 12 months of age—effects of body size, growth and diet in a population with high birth weight. *Eur J Clin Nutr*. 2003;57(4): 505–513
  15. Nordic Expert Group for Nutritional Recommendations. *Nordic Nutrition Recommendation 2004*. Copenhagen, Denmark: Nordic Council of Ministers, 2004:13
  16. Michaelsen KF, Milman N, Samuelson G. A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors. *Acta Paediatr*. 1995;84(9):1035–1044
  17. Fuchs GJ, Farris RP, DeWier M, et al. Iron status and intake of older infants fed formula vs cow milk with cereal. *Am J Clin Nutr*. 1993;58(3):343–348
  18. Beard JL, Murray-Kolb LE, Rosales FJ, Solomons NW, Angelilli ML. Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: the role of biomarkers for the acute phase response. *Am J Clin Nutr*. 2006; 84(6):1498–1505
  19. Singer JL, Buchino JJ, Chabali R. Selected laboratory in pediatric emergency care. *Emerg Med Clin North Am*. 1986;4(2):377–396
  20. Wells JC, Jonsdóttir OH, Hibberd PL, et al. Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: differences in breast-milk intake by stable-isotope probe. *Am J Clin Nutr*. 2012; 96(1):73–79
  21. Ceriani Cernadas JM, Carroli G, Pellegrini L, et al. The effect of early and delayed umbilical cord clamping on ferritin levels in term infants at six months of life: a randomized, controlled trial [in Spanish]. *Arch Argent Pediatr*. 2010;108(3):201–208
  22. Hutton EK, Hassan ES. Late vs early clamping of the umbilical cord in full-term neonates: systematic review and meta-analysis of controlled trials. *JAMA*. 2007; 297(11):1241–1252
  23. Chaparro CM, Neufeld LM, Tena Alavez G, Eguia-Liz Cedillo R, Dewey KG. Effect of timing of umbilical cord clamping on iron status in Mexican infants: a randomised controlled trial. *Lancet*. 2006;367(9527): 1997–2004
  24. Dewey KG, Cohen RJ, Rivera LL, Brown KH. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. *Am J Clin Nutr*. 1998;67 (5):878–884
  25. Yang Z, Lönnardal B, Adu-Afarwuah S, et al. Prevalence and predictors of iron deficiency in fully breastfed infants at 6 mo of age: comparison of data from 6 studies. *Am J Clin Nutr*. 2009;89(5):1433–1440
  26. Wharf SG, Fox TE, Fairweather-Tait SJ, Cook JD. Factors affecting iron stores in infants 4–18 months of age. *Eur J Clin Nutr*. 1997; 51(8):504–509
  27. Lartey A, Manu A, Brown KH, Dewey KG. Predictors of micronutrient status among six- to twelve-month-old breast-fed Ghanaian infants. *J Nutr*. 2000;130(2):199–207
  28. Siegenberg D, Baynes RD, Bothwell TH, et al. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phy-tates on nonheme-iron absorption. *Am J Clin Nutr*. 1991;53(2):537–541

(Continued from first page)

[www.pediatrics.org/cgi/doi/10.1542/peds.2011-3838](http://www.pediatrics.org/cgi/doi/10.1542/peds.2011-3838)

doi:10.1542/peds.2011-3838

Accepted for publication Jul 27, 2012

Address correspondence to Ólof H. Jonsdóttir, BSc, Unit for Nutrition Research, Landspítali National University Hospital of Iceland, Eiríksgrata 29; IS-101 Reykjavík, Iceland. E-mail: [olofhej@landspitali.is](mailto:olofhej@landspitali.is)

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2012 by the American Academy of Pediatrics

**FINANCIAL DISCLOSURE:** Drs Fewtrell and Lucas have received research funding from and undertaken advisory work for companies manufacturing infant foods and feeding products within the past 3 years; the other authors have indicated they have no financial relationship relevant to this article to disclose.

**FUNDING:** This study was supported by Mead Johnson and the Eimskip Fund of the University of Iceland. Additional support was provided by the Primary Health Care Organisations in Reykjavík Capital Area, Akranes and Sudurnes, and the directors of the participating health centers.

## Timing of the Introduction of Complementary Foods in Infancy: A Randomized Controlled Trial

Olof H. Jonsdottir, Inga Thorsdottir, Patricia L. Hibberd, Mary S. Fewtrell, Jonathan C. Wells, Gestur I. Palsson, Alan Lucas, Geir Gunnlaugsson and Ronald E. Kleinman

*Pediatrics* 2012;130;1038; originally published online November 12, 2012;

DOI: 10.1542/peds.2011-3838

<b>Updated Information &amp; Services</b>	including high resolution figures, can be found at: <a href="http://pediatrics.aappublications.org/content/130/6/1038.full.html">http://pediatrics.aappublications.org/content/130/6/1038.full.html</a>
<b>References</b>	This article cites 22 articles, 10 of which can be accessed free at: <a href="http://pediatrics.aappublications.org/content/130/6/1038.full.html#ref-list-1">http://pediatrics.aappublications.org/content/130/6/1038.full.html#ref-list-1</a>
<b>Subspecialty Collections</b>	This article, along with others on similar topics, appears in the following collection(s): <b>Nutrition &amp; Metabolism</b> <a href="http://pediatrics.aappublications.org/cgi/collection/nutrition_and_metabolism">http://pediatrics.aappublications.org/cgi/collection/nutrition_and_metabolism</a> <b>Gastrointestinal Tract</b> <a href="http://pediatrics.aappublications.org/cgi/collection/gastrointestinal_tract">http://pediatrics.aappublications.org/cgi/collection/gastrointestinal_tract</a>
<b>Permissions &amp; Licensing</b>	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: <a href="http://pediatrics.aappublications.org/site/misc/Permissions.xhtml">http://pediatrics.aappublications.org/site/misc/Permissions.xhtml</a>
<b>Reprints</b>	Information about ordering reprints can be found online: <a href="http://pediatrics.aappublications.org/site/misc/reprints.xhtml">http://pediatrics.aappublications.org/site/misc/reprints.xhtml</a>

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2012 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™



# Paper III

**Title:**

Exclusive breastfeeding and developmental and behavioral status in early childhood

**Authors:**

Olof H Jonsdottir, BSc<sup>1</sup>, Inga Thorsdottir, PhD<sup>1</sup>, Geir Gunnlaugsson, MD, PhD<sup>2</sup>, Mary S Fewtrell, MD<sup>3</sup>, Patricia L Hibberd, MD, PhD<sup>4</sup>, Ronald E Kleinman, MD<sup>5</sup>

**Affiliations:**

<sup>1</sup>Unit for Nutrition Research, Landspítali - The National University Hospital of Iceland and Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, Reykjavik, Iceland.

<sup>2</sup>Directorate of Health and Reykjavik University, Iceland.

<sup>3</sup>Childhood Nutrition Research Centre, UCL Institute of Child Health, London, UK.

<sup>4</sup>Division of Global Health, Massachusetts General Hospital for Children, Harvard Medical School, Boston, USA.

<sup>5</sup>Department of Pediatrics, Massachusetts General Hospital for Children, Harvard Medical School, Boston, MA, USA.

**Address correspondence to:**

Olof H Jonsdottir, Unit for Nutrition Research, Landspítali National University Hospital of Iceland, Eiríksgata 29; IS-101 Reykjavik, Iceland, Telephone: +354 543 1452, Fax: +354 543 4824, E-mail: [ohj@hi.is](mailto:ohj@hi.is)

**Short title:** Exclusive breastfeeding and developmental status

**Abbreviations:** CF - Complementary feeding in addition to breast milk from the age of 4 months; EBF - Exclusive breastfeeding to the age of 6 months; PEDS - Parent's Evaluation of Developmental Status

**Key Words:** Exclusive breastfeeding, developmental status, randomized trial

**Funding Source:** This study was supported by Mead Johnson and the Eimskip Fund of the University of Iceland. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, preparation of the report or the decision to submit for publication.

**Clinical Trial Registration:** ISRCTN41946519

### **Contributor's Statement:**

Olof H Jonsdottir: Ms. Jonsdottir was responsible for the data collection and, following extensive discussions amongst the study team, conducted the statistical analysis and wrote the first draft of the manuscript and was responsible for the final decision to submit the manuscript.

Inga Thorsdottir: Professor Thorsdottir was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Geir Gunnlaugsson: Dr. Gunnlaugsson was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Mary S Fewtrell: Dr. Fewtrell was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Patricia L Hibberd: Dr. Hibberd was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Ronald E Kleinman: Dr. Kleinman was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

### **Acknowledgements**

The authors greatly acknowledge all participants in the study and the directors and staff at the participating health centers for support to conduct the study. In particular, the nurses Sesselja Gudmundsdottir, Dagny Gudmundsdottir (HC Hvammur), Audur Egilsdottir (HC Hamraborg) and Kristin J. Vigfusdottir (HC Grafarvogur) and the nurses and the lactation consultants Jona Margret Jonsdottir (Centre for Preventive Child Health Services), Alma Maria Rognvaldsdottir (HC Fjordur), Elin Sigurbjornsdottir (HC Akranes), Gudrun Gudmundsdottir (HC Sudurnes) and Ingibjorg Eiriksdottir (HC Grafarvogur) and for recruiting mother-infant pairs and their great amount of assistance in data collection. Furthermore we gratefully acknowledge Dr. Frances Page Glascoe for constructive comments on earlier drafts of the paper.

## **ABSTRACT**

Breastfeeding during infancy may have beneficial effects on various developmental outcomes in childhood. In this study 119 exclusively breastfed infants were randomly assigned to receive complementary foods from 4 months of age in addition to breast milk, or to exclusively breastfeed to 6 months. At 18 and 30-35 months, the children were evaluated with the Parent's Evaluation of Developmental Status questionnaire (PEDS) and the Brigance Screens-II at 30-35 months. No sustained effect of a longer duration of exclusive breastfeeding was seen on selected measures of developmental and behavioral status at 18 months, although at 30-35 months, a smaller percentage of parents of children introduced to complementary foods at 4 months of age expressed concerns about their gross motor development.

Breastfeeding may have beneficial effects on cognitive development in childhood, adolescence and even in adulthood (Horta, Bahl, Martinés & Victoria, 2007; Schack-Nielsen & Michaelsen, 2007), although this has not been a consistent finding (Ip et al., 2007). Furthermore, some studies indicate that a longer duration of exclusive breastfeeding is important for this positive association with developmental outcomes in childhood, especially for those born small for gestational age (Kramer & Aboud et al., 2008; Jedrychowski et al., 2012; Rao et al., 2002). Less is known about the impact of breastfeeding and the duration of exclusive breastfeeding on non-cognitive developmental and behavioral status in childhood. Studies indicate that breastfeeding in general and also longer duration of breastfeeding may be associated with decreased risk of behavioral problems and developmental delays in childhood (Oddy et al., 2010; Halpern et al., 2000; Paterson, Iusitini & Gao, 2011). However findings on this subject are inconsistent. A large breastfeeding promotion intervention in Belarus showed no relationship between prolonged breastfeeding or longer duration of exclusive breastfeeding and childrens' behavior at 6.5 years of age (Kramer & Fombonne et al., 2008). Other studies have shown that increased duration and exclusivity of breastfeeding may have beneficial effects on language and motor development in childhood (Chiu et al., 2011; Whitehouse, Robinson, Li & Oddy, 2011; Quinn et al., 2001; Tozzi et al., 2012; Dee, Li, Lee & Grummer-Strawn, 2007; Vestergaard et al., 1999; Thorsdottir, Gunnarsdottir, Kvaran & Gretarsson, 2005).

We have previously reported the results of a parallel group, masked, randomized controlled trial of the effects of exclusive breastfeeding for 4 vs. 6 months on growth, body composition, breast-milk intake and iron status of the infant (Wells et al., 2012; Jonsdottir et al., 2012). We now report a secondary analysis from this cohort of exclusive breastfeeding infants for 4 vs. 6 months on selected measures of development and behavior in early

childhood. We hypothesized that infants exclusively breastfed for 6 months would have better outcomes in selected measures of developmental and behavioral status than those receiving complementary foods from 4 months in addition to breast milk.

## **METHODS**

### **Study design**

As described previously (Wells et al., 2012; Jonsdottir et al., 2012), between November 2007 and November 2009 a total of 119 mother-infant pairs were recruited at 7 health care centers in Iceland where 50% and 35% of mothers exclusively breastfeed through 4 and 5 months of age, respectively (Directorate of Health, 2012). Eligibility criteria for the study were singleton birth, gestational length  $\geq 37$  weeks, exclusively breastfed, infant characterized as healthy: absence of congenital abnormalities or chronic health issues likely to affect growth, development or iron status. Mothers of eligible infants were invited to participate in the study and infants who were still exclusively breastfed and whose parents were willing to participate were enrolled in the study at 4 months of age. Eligible mother-infant pairs were randomly assigned to receive complementary foods from the age of 4 months in addition to breast milk (CF), or to continue being exclusively breastfed to the age of 6 months (EBF). Vitamin D supplements were recommended in both groups. Exclusive breastfeeding was defined as breastfeeding with no additional liquid or solid foods other than vitamins and medications (WHO, 1991). The use of up to 10 feedings of formula or water during the first 6 months was allowed to avoid having to exclude infants that in fact were otherwise exclusively breastfed.

The study was reviewed and approved by the Data Protection Authority and National Bioethical Committee in Iceland and the Partners Health System IRB, Boston, MA.



## **Selected measures of developmental and behavioral status**

Children in the present study were assessed both at 18 months and 29-38 months of age, during their routine health care visits at the health center, where developmental and behavioral status was assessed with both the Parent's Evaluation of Developmental Status (PEDS) questionnaire and the Brigance Screens-II. The parents filled out the PEDS questionnaire at both visits, at 18 months and 30-35 months of age, and the children underwent the Brigance Screens-II at 30-35 months. Both tests were administered by trained nurses at each health care center following prescribed protocols (Glascoe, 2008; Glascoe, 2009; Gunnlaugsson & Saemundsson, 2005). PEDS questionnaire and Brigance Screens-II were both introduced in 2010 as a part of routine health care visits at health centers in Iceland.

The PEDS is designed to detect parental concerns about the developmental status and behaviors of their child; it has been found to have very good reliability and has been validated for children from birth to 8 years of age (Glascoe, 1999; Glascoe, 2000; Glascoe, 2000; Rydz, Shevell, Majnemer & Oskoui, 2005; Brothers, Glascoe & Robertshaw, 2008). The PEDS questionnaire consists of 10 brief questions, 2 open-ended about general cognitive function and other concerns and 8 domain-specific items. For each of the 8 domain-specific questions the parents are asked if they have any concerns about the development or behavior of their child and their response option is in a multiple-choice format (no, yes, a little). Certain parental expressions of concern in response to certain of these questions are predictive of developmental delay (Glascoe, 2008). If parents express concern in response to  $\geq 2$  of these predictive questions then health center procedures require that the child be referred for further evaluation (see **Figure 1**). It takes parents approximately 5 minutes to answer the questionnaire.

The Brigance Screens-II is administered by a trained nurse who observes the child and questions his/her parents and the test is completed by the child itself. It has good reliability and has been validated for measuring the developmental and behavioral status of toddlers and preschool children (Glascoe, 2005; Glascoe, 2001; Glascoe, 2002). The Brigance Screens-II for 30-35 month old children is valid for children from the age of 29 months+15 days to 35 months+14 days old children. The Brigance Screens-II comprises 11 components and it takes children approximately 15-20 minutes to complete the test. The cut off points for defining children at risk of developmental delay are <72 and <76 points of 100 points for children aged 30-32 months and 33-35 months, respectively. As with the PEDS questionnaire, there are some components of the Brigance Screens-II more predictive for developmental delay than others among all the test components (see **Figure 1**). Cut off points for defining children at risk of developmental delay are <34 and <35 points for children aged 30-32 and 33-35 months, respectively (Directorate of Health and Primary Health Care Organisation, 2010). In the current study we focused on assessment of gross motor skills (3B), fine motor skills (6B, 8B) and receptive or expressive language (5B, 10B, 11B), since studies indicate that breastfeeding may influence these factors. (Chiu et al., 2011; Whitehouse, Robinson, Li & Oddy, 2011; Quinn et al., 2001; Tozzi et al., 2012; Dee, Li, Lee & Grummer-Strawn, 2007; Vestergaard et al., 1999; Thorsdottir, Gunnarsdottir, Kvaran & Gretarsson, 2005).

### **Statistical analysis**

Data were analyzed with SPSS Windows statistical software package version 20.0 (SPSS Inc., Chicago, IL, USA) with a level of significance of  $p \leq 0.05$ . Data were presented with means and standard deviations (SD) for normally distributed variables and with median and interquartile range (IQR) for variables with skewed distribution. Group comparisons were performed using independent-samples t-test and Mann-Whitney U-test. Comparisons between

categorical values were made using the Chi-square tests of association or two-sided Fisher's exact test. Regression analysis was performed to adjust for any pre-randomization characteristics that were different between the two intervention groups at baseline. Finally, we calculated the power to detect differences between the CF and EBF groups based on proportions.

## **RESULTS**

### **Sample size and characteristics of participants**

Of the 100 mother-infant pairs who finished the breastfeeding intervention trial, a total of 95 children attended routine care at 18 months and 82 at 30-35 months. 54 parents answered the PEDS questionnaire when their child was 18 months and 78 parents at the 30-35 months visit. Since both PEDS questionnaire and Brigance Screens-II were introduced in 2010 we have 41 missing data points from PEDS questionnaire at 18 months of age for those children in the study born in 2007 and some who were born in 2008. Parents of 4 children who attended routine care at 30-35 months did not answer the PEDS questionnaire at that age. The Brigance Screens-II was undertaken by 77 children at the age of 30-35 months, but 10 of them were too old ( $>35$  months+14 days) and 1 too young ( $<29$  months+15 days) when the Brigance Screens-II was performed and were therefore excluded from the analysis. The PEDS questionnaire is for wider age range but we chose to use 30-35 months throughout the paper. The children that did not have developmental scores recorded from the Brigance Screens-II ( $n=23$ ) were lost to follow-up for several reasons, such as the family had moved abroad or failure to attend the routine health care visits at the health center.

Among those children with developmental scores from the Brigance Screens-II at 30-35 months of age (n=66), no differences between study groups were seen in baseline characteristics, except for mode of delivery, where vaginal delivery was more common among children in the CF group (94% vs. 74% in the EBF group,  $p=0.04$ ) (see **Table 1**). No difference was seen in baseline characteristics (same as seen in Table 1) among those who were followed-up (n=82) and those who were lost to follow-up (n=18), except for parity, where those parents who were lost to follow-up had more children ( $3.0\pm1.0$  children) than those who were followed-up ( $2.0\pm2.0$  children;  $p=0.01$ ).

### **Developmental and behavioral status**

**Table 2** shows the developmental and behavioral status measures in the two study groups at 18 months (PEDS questionnaire) and at 30-35 months (PEDS questionnaire and Brigance Screens-II). At 18 months a significantly smaller percentage of parents had concerns about any of the domains of PEDS on their children's developmental and behavioral status in the CF group compared with those in the EBF group (17% in the CF group vs. 44% in the EBF group;  $p=0.03$ ). A logistic regression was done to test the impact of the intervention by group and when adjusted for mode of delivery the difference in parents' concerns between groups at 18 months was not statistically significant ( $p=0.08$ ). No difference was seen between groups in number of concerns regarding gross or fine motor skills or receptive and expressive language. At 18 months parents most often expressed concerns about their children's expression and articulation of the 8 domain-specific questions, 10% and 20% in the CF and EBF group, respectively ( $p=0.45$ ). No significant differences were seen even when those questions with greater predictive value for developmental delay were compared among groups at 18 months (0% in the EBF group vs. 3% in the CF group;  $p=1.0$ ).

At 30-35 months of age no significant differences were seen between study groups in number of parents with concerns about any of the domains of PEDS (42% in the EBF group vs. 33% in the CF group;  $p=0.45$ ). A smaller proportion of parents of children in the CF group (2%) had concerns about their gross motor development compared with parents of those in the EBF group (19%;  $p=0.01$ ). When adjusted for mode of delivery the difference was still significant ( $p=0.03$ ). No difference was seen between groups in number of concerns regarding fine motor skills or receptive and expressive language. At 30-35 months parents most often expressed their concerns about their children's expression and articulation of the 8 domain-specific questions, 19% and 28% in the CF and EBF group, respectively ( $p=0.36$ ). Use of the cut off of  $\geq 2$  predictive concerns for PEDS questionnaire at 30-35 months showed that 19% of parents in the EBF group were above the cut off value compared with 5% of the parents in the CF group, although the difference was not significant ( $p=0.07$ ).

There was no significant difference between study groups at 30-35 months by the Brigance Screens-II ( $p=0.82$ ). Neither was there a significant difference between groups in number of children below the cut off value defining developmental delays for total score from the Brigance Screens-II ( $p=0.41$ ) or number of children above the cut off value defining developmental delays from predictive components of the Brigance screening test combined ( $p=0.32$ ). Furthermore there was no significant difference between groups in fine or gross motor skills or receptive and expressive language according to the Brigance Screens-II at 30-35 months. Excluding 3 outliers found in the EBF group in the Brigance screening test did not change the mean values for the study groups or the lack of significance ( $86.1 \pm 7.8$  points in the CF group vs.  $88.0 \pm 7.4$  points in the EBF group;  $p=0.33$ ). To detect a significant difference between intervention groups in developmental scores from the Brigance screening test at 30-35 months of age with a sample size of 66 and a power of 80%, the mean difference in

developmental scores would have had to be approximately 11.2 points, or approximately 5.4 points if excluding the three outliers (n=63).

## **DISCUSSION**

In this study, of well-nourished children, at 30-35 months of age, a smaller proportion of parents in the CF group expressed their concerns about their children's gross motor development on the PEDS questionnaire, a difference that remained significant when adjusted for differences in pre-randomization characteristics. However, there were no significant intergroup differences in developmental total scores or in fine and gross motor skills or receptive and expressive language according to the Brigance Screens-II at 30-35 months. No difference was seen in the percentage of parents with concerns about their children's developmental and behavioral status at the age of 18 months.

Results from the PEDS questionnaire are based on a small number of categorical variables. Outcomes from the Brigance Screens-II however are based on continuous variables and therefore this test is more responsive to detecting minor developmental disabilities. The Brigance Screens-II is a comprehensive, reliable and valid screening tool of developmental status that is completed by the child itself (Glascoe, 2002; American Academy of Pediatrics, 2006). Similar general developmental screening tools that are directly administered to the child and are used in primary care settings are the Battelle Developmental Inventory Screening Tool Test II, the Bayley Infant Neurodevelopmental Screener and the Denver-II Developmental Screening Test, which are all comparable to the Brigance Screens-II in sensitivity and specificity (American Academy of Pediatrics, 2006; Glascoe, 2007). Per health center protocols in the Icelandic healthcare system, children identified at risk for

developmental delay or behavioral problems according to the Brigance Screens-II or the PEDS questionnaire are referred for further evaluation, diagnosis and then developmental intervention, if appropriate. Early detection of developmental delay and appropriate intervention has been shown to be effective in improving developmental outcomes in childhood (Anderson et al., 2003).

Although the PEDS is solely based on parental perception of their children's developmental and behavioral status, a positive correlation has been shown between the results of the PEDS questionnaire and the Brigance Screens-II (Gunnlaugsson & Saemundsson, 2005). The PEDS questionnaire is a valid and reliable developmental screening tool (American Academy of Pediatrics, 2006) and the value of parents' concerns in the detection of developmental delay has been well studied (Glascoe, 2000; Glascoe, 2003). Comparable commonly used parent-completed screening questionnaires in primary care settings comparable are the Ages & Stages Questionnaires, the Child Development Review-Parent Questionnaire and the Infant Development Inventory (American Academy of Pediatrics, 2006; Glascoe, 2007). These tests are not perfectly concordant, but are widely used and are considered appropriate for developmental evaluation in primary care settings (Sices, Stancin, Kirchner & Bauchner, 2009; Limbos & Joyce, 2011). It should be noted, that although some parental concerns are predictive over time, the PEDS questionnaire does not always capture longitudinal changes in developmental status since parents may have fewer concerns after their child begins a developmental intervention, even though developmental delays may still be present. In 2006, the American Academy of Pediatrics recommended systematic developmental screening in primary care using a validated screening tool for children aged 9, 18 and 30 months, but no specific guidance was provided for the specific screening tools that should be used (American Academy of Pediatrics, 2006). Health care

centers in Iceland chose to use the PEDS questionnaire and the Brigance screening test because of their good reliability and validity and well-established sensitivity and specificity and because they are useable for a wide range of ages in childhood (Gunnlaugsson & Saemundsson, 2005).

To our knowledge this is the first secondary analysis of a randomized controlled trial conducted in a resource rich country to examine the effects of exclusive breastfeeding for 4 vs. 6 months on selected measures of developmental and behavioral status in early childhood. The World Health Organization (WHO) recommended exclusive breastfeeding for 4-6 months of life until the year 2001 when the recommendation was changed to breastfeed exclusively for the first 6 months of life in an effort to lower the risk of adverse health outcomes for infants during the first 6 months, particularly in resource constrained countries (Kramer, Chalmers, Hodnett et al., 2001). Developmental status is influenced by a number of genetic and environmental factors that cause cumulative risk effects of development delays that are generally not addressed in observational studies. This is one possible explanation for the inconsistent findings among such studies (Whitehouse, Robinson, Li & Oddy, 2011; Zhou, Baghurst, Gibson & Makrides, 2007; Quigley et al., 2012). Studies investigating the relationship between breastfeeding and developmental status often compare formula fed infants to breastfed infants (Quigley et al., 2012; Anderson, Johnstone & Remley, 1999; Fewtrell et al., 2002; Slykerman et al., 2005), but less is known about the impact of exclusive breastfeeding compared to partial breastfeeding.

There is strong evidence that nutrition early in life can have long-term effects on health and development later in life (Koletzko et al., 2012; Turck, 2007; Wu & Chen, 2009). It has been suggested that the concentration of long chain polyunsaturated fatty acids in breast milk may explain the enhanced cognitive outcomes reported in some studies comparing



breastfed and formula fed infants (Helland et al., 2003; Mitmesser & Jensen, 2007; Koletzko & Rodriguez-Palmero, 1999; Lanting et al., 1994) and therefore the effect of duration of exclusive breastfeeding on developmental and behavioral status would also be a relevant factor in these outcomes. Since infants exclusively breastfed for 6 months in the present study had significantly higher breast milk intakes at 5.5-6 months of age (Wells et al., 2012) we hypothesized they would have better developmental and behavioral status in early childhood. However, no intergroup differences in measures of developmental and behavioral status outcomes were observed among those that completed the Brigance Screens-II at 30-35 months of age. The parental impressions from the PEDS questionnaire administered when the children were 30-35 months of age were thus not substantiated by the more objective and reliable Brigance Screens-II at the same age. The reason for no difference in these developmental and behavioral measures might be because both study groups consumed a significant amount of breast milk. While the infants in the EBF group consumed a mean of 83 g/d more of breast milk than those in the CF group (Wells et al., 2012), the amount consumed by the CF group was consistent with the recommendations of the WHO (Butte, Lopez-Alarcon & Garza, 2002).

The strength of the present study is the fact that this is the only analysis of later developmental and behavioral data from a randomized controlled trial of 4 vs. 6 months of exclusive breastfeeding and it therefore has a methodological advantage over previously published observational studies. Furthermore, approximately 82% of the cohort was follow-up until the age of 30-35 months. The main limitation of the present study is that data were collected in routine health care visits at the health center. We recognize that this secondary data analysis may have been underpowered to detect small effects on developmental and behavioral outcomes that may be biologically relevant, but the sample size was adequate to

exclude large effects on developmental and behavioral outcomes in the 2 groups (Fewtrell et al., 2008).

In addition to breast milk per se, other factors that influence infant development may have played a role in the outcomes we observed in this randomized trial. Infants with depleted iron stores, iron deficiency or iron deficiency anemia can have lower developmental scores in childhood (Iannotti, Tielsch, Black M & Black R, 2006; Gunnarsson, Thorsdottir, Palsson & Gretarsson, 2007), however we have previously reported that no differences was seen in the prevalence of iron deficiency with or without anemia between both groups at 6 months of age.<sup>(19)</sup> Mothers who choose to breastfeed may differ from those who never breastfeed in many ways that can influence an infant's development, including socio-economic status and nurturing qualities. However mothers in both study groups exclusively breastfed for the first 4 months of their infant's life all were from a similar socioeconomic background and thereafter all of them continued breastfeeding partially or exclusively until 6 months of age or beyond, minimizing the impact of these other influential developmental factors.

In conclusion, the present study showed no sustained effect of a longer duration of exclusive breastfeeding on selected measures of developmental and behavioral status at 18 months of age although at 30-35 months, a smaller percentage of parents of infants introduced to complementary foods at 4 months of age expressed concerns about their children's gross motor development. Further investigation is needed in a larger randomized controlled trial using the same or other measures of developmental and behavioral status to extend and confirm these findings.

## REFERENCES

- American Academy of Pediatrics, Council on Children With Disabilities; Section on Developmental Behavioral Pediatrics; Bright Futures Steering Committee; Medical Home Initiatives for Children With Special Needs Project Advisory Committee. Identifying infants and young children with developmental disorders in the medical home: an algorithm for developmental surveillance and screening. *Pediatrics*. 2006;118(1):405-20.
- Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr*. 1999;70(4):525-35.
- Anderson LM, Shinn C, Fullilove MT, Scrimshaw SC, Fielding JE, Normand J, Carande-Kulis VG; Task Force on Community Preventive Services. The effectiveness of early childhood development programs. A systematic review. *Am J Prev Med*. 2003;24(3 Suppl):32-46.
- Brothers KB, Glascoe FP, Robertshaw NS. PEDS: developmental milestones--an accurate brief tool for surveillance and screening. *Clin Pediatr (Phila)*. 2008;47(3):271-9.
- Butte NF, Lopez-Alarcon MG, Garza C. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. Geneva: World Health Organisation; 2002.
- Chiu WC, Liao HF, Chang PJ, Chen PC, Chen YC. Duration of breast feeding and risk of developmental delay in Taiwanese children: a nationwide birth cohort study. *Paediatr Perinat Epidemiol*. 2011;25(6):519-27.
- Dee DL, Li R, Lee LC, Grummer-Strawn LM. Associations between breastfeeding practices and young children's language and motor skill development. *Pediatrics*. 2007;119 Suppl 1:S92-8.
- Directorate of Health and Primary Health Care Organisation of Greater Reykjavik Area. Ung- og smábarnavernd. Leiðbeiningar um heilsuvernd barna 0-5 ára (Preventive child health services: Guidelines for preventive health services for children 0-5 years of age). Eds.: Guðmundsdóttir S Aradóttir AB, Gunnlaugsson G. Reykjavík: The Organisations 2010. Available at <http://www.landlaeknir.is/servlet/file/store93/item2830/version6/4138.pdf> Accessed October 23<sup>rd</sup>, 2012.
- Directorate of Health. Brjóstagjöf og næring ungbarna á Íslandi sem fædd eru 2004-2008 (Breastfeeding and infant nutrition among infants born 2004-2008 in Iceland). 2012. Available at [http://www.landlaeknir.is/servlet/file/store93/item16573/version4/brjostagjof\\_og\\_naering\\_2004-2008\\_juni.2012.pdf](http://www.landlaeknir.is/servlet/file/store93/item16573/version4/brjostagjof_og_naering_2004-2008_juni.2012.pdf) Accessed November 11<sup>th</sup>, 2012.
- Fewtrell MS, Kennedy K, Singhal A, Martin RM, Ness A, Hadders-Algra M, Koletzko B, Lucas A. How much loss to follow-up is acceptable in long-term randomised trials and prospective studies? *Arch Dis Child*. 2008;93(6):458-61.

Fewtrell MS, Morley R, Abbott RA, Singhal A, Isaacs EB, Stephenson T, MacFadyen U, Lucas A. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. *Pediatrics*. 2002;110(1 Pt 1):73-82.

Glascoe FP, Squires J. Issues with the new developmental screening and surveillance policy statement. *Pediatrics*. 2007;119(4):861-2; discussion 862-3.

Glascoe FP. Are overreferrals on developmental screening tests really a problem? *Arch Pediatr Adolesc Med*. 2001;155(1):54-9.

Glascoe FP. Detecting and addressing developmental and behavioral problems in primary care. *Pediatr Nurs*. 2000;26(3):251-7.

Glascoe FP. Evidence-based approach to developmental and behavioural surveillance using parents' concerns. *Child Care Health Dev*. 2000;26(2):137-49.

Glascoe FP. Parents' evaluation of developmental status: how well do parents' concerns identify children with behavioral and emotional problems? *Clin Pediatr (Phila)*. 2003;42(2):133-8.

Glascoe FP. Screening for developmental and behavioral problems. *Ment Retard Dev Disabil Res Rev*. 2005;11(3):173-9.

Glascoe FP. The Brigance Infant and Toddler Screen: standardization and validation. *J Dev Behav Pediatr*. 2002;23(3):145-50.

Glascoe FP. Using parents' concerns to detect and address developmental and behavioral problems. *J Soc Pediatr Nurs*. 1999;4(1):24-35.

Glascoe, F.P. PEDS, Mat foreldra á þroska barna (Parental perception on their child's developmental outcomes). 2009. Reykjavik: Educational Testing Institute and Directorate of Health.

Glascoe, F.P. Technical Report for the BRIGANCE SCREENS. 2008. Reykjavik: Educational Testing Institute and Directorate of Health.

Gunnarsson BS, Thorsdottir I, Palsson G, Gretarsson SJ. Iron status at 1 and 6 years versus developmental scores at 6 years in a well-nourished affluent population. *Acta Paediatr*. 2007;96(3):391-5.

Gunnlaugsson G, Saemundsson E. Að finna frávik í þroska og hegðun fimm ára barna (Finding deviation in development and behaviour of 5 year old children). Í *Ungir Íslendingar í ljósi vísindanna*, p.237-245. 2005. Reykjavik: Representative for children and the University of Iceland. 2005.

Halpern R, Giugliani ER, Victora CG, Barros FC, Horta BL. Risk factors for suspicion of developmental delays at 12 months of age. *J Pediatr (Rio J)*. 2000;76(6):421-8.

- Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics*. 2003;111(1):e39-44.
- Horta BL, Bahl R, Martinés JC, Victora CG. Evidence on the long-term effects of breastfeeding: Systematic reviews and meta-analyses. Geneva, Switzerland: World Health Organization; 2007.
- Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. *Am J Clin Nutr*. 2006;84(6):1261-76.
- Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T, Lau J. Breastfeeding and maternal and infant health outcomes in developed countries. *Evid Rep Technol Assess* (Full Rep). 2007;(153):1-186.
- Jedrychowski W, Perera F, Jankowski J, Butscher M, Mroz E, Flak E, Kaim I, Lisowska-Miszczyk I, Skarupa A, Sowa A. Effect of exclusive breastfeeding on the development of children's cognitive function in the Krakow prospective birth cohort study. *Eur J Pediatr*. 2012;171(1):151-8.
- Jonsdottir OH, Thorsdottir I, Hibberd PL, Fewtrell MS, Wells JC, Palsson GI, Lucas A, Gunnlaugsson G, Kleinman RE. Timing of the introduction of complementary foods in infancy: A randomized controlled trial. *Pediatrics*. 2012;130(6):1038-45.
- Koletzko B, Brands B, Poston L, Godfrey K, Demmelmair H. Early nutrition programming of long-term health. *Proc Nutr Soc*. 2012;71(3):371-8.
- Koletzko B, Rodriguez-Palmero M. Polyunsaturated fatty acids in human milk and their role in early infant development. *J Mammary Gland Biol Neoplasia*. 1999;4(3):269-84.
- Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, Igumnov S, Fombonne E, Bogdanovich N, Ducruet T, Collet JP, Chalmers B, Hodnett E, Davidovsky S, Skugarevsky O, Trofimovich O, Kozlova L, Shapiro S; Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group. Breastfeeding and child cognitive development: new evidence from a large randomized trial. *Arch Gen Psychiatry*. 2008;65(5):578-84.
- Kramer MS, Chalmers B, Hodnett ED et.al. Promotion of Breastfeeding Intervention Trial (PROBIT): A Randomized Trial in the Republic of Belarus. *JAMA* 2001;285:413-20.
- Kramer MS, Fombonne E, Igumnov S, Vanilovich I, Matush L, Mironova E, Bogdanovich N, Tremblay RE, Chalmers B, Zhang X, Platt RW; Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group. Effects of prolonged and exclusive breastfeeding on child behavior and maternal adjustment: evidence from a large, randomized trial. *Pediatrics*. 2008;121(3):e435-40.
- Lanting CI, Fidler V, Huisman M, Touwen BC, Boersma ER. Neurological differences between 9-year-old children fed breast-milk or formula-milk as babies. *Lancet*. 1994;344(8933):1319-22.

- Limbos MM, Joyce DP. Comparison of the ASQ and PEDS in screening for developmental delay in children presenting for primary care. *J Dev Behav Pediatr*. 2011;32(7):499-511.
- Mitmesser SH, Jensen CL. Roles of long-chain polyunsaturated fatty acids in the term infant: developmental benefits. *Neonatal Netw*. 2007;26(4):229-34.
- Oddy WH, Kendall GE, Li J, Jacoby P, Robinson M, de Klerk NH, Silburn SR, Zubrick SR, Landau LI, Stanley FJ. The long-term effects of breastfeeding on child and adolescent mental health: a pregnancy cohort study followed for 14 years. *J Pediatr*. 2010;156(4):568-74.
- Paterson J, Iusitini L, Gao W. Child developmental assessment at two-years of age: data from the Pacific Islands Families Study. *Pac Health Dialog*. 2011;17(2):51-63.
- Quigley MA, Hockley C, Carson C, Kelly Y, Renfrew MJ, Sacker A. Breastfeeding is associated with improved child cognitive development: a population-based cohort study. *J Pediatr*. 2012;160(1):25-32.
- Quinn PJ, O'Callaghan M, Williams GM, Najman JM, Andersen MJ, Bor W. The effect of breastfeeding on child development at 5 years: a cohort study. *J Paediatr Child Health*. 2001;37(5):465-9.
- Rao MR, Hediger ML, Levine RJ, Naficy AB, Vik T. Effect of breastfeeding on cognitive development of infants born small for gestational age. *Acta Paediatr*. 2002;91(3):267-74.
- Rydz D, Shevell MI, Majnemer A, Oskoui M. Developmental screening. *J Child Neurol*. 2005;20(1):4-21.
- Schack-Nielsen L, Michaelsen KF. Advances in our understanding of the biology of human milk and its effects on the offspring. *J Nutr*. 2007;137(2):503S-510S.
- Sices L, Stancin T, Kirchner L, Bauchner H. PEDS and ASQ developmental screening tests may not identify the same children. *Pediatrics*. 2009;124(4):e640-7.
- Slykerman RF, Thompson JM, Becroft DM, Robinson E, Pryor JE, Clark PM, Wild CJ, Mitchell EA. Breastfeeding and intelligence of preschool children. *Acta Paediatr*. 2005;94(7):832-7.
- Thorsdottir I, Gunnarsdottir I, Kvaran MA, Gretarsson SJ. Maternal body mass index, duration of exclusive breastfeeding and children's developmental status at the age of 6 years. *Eur J Clin Nutr*. 2005;59(3):426-31.
- Tozzi AE, Bisiacchi P, Tarantino V, Chiarotti F, D'Elia L, De Mei B, Romano M, Gesualdo F, Salmaso S. Effect of duration of breastfeeding on neuropsychological development at 10 to 12 years of age in a cohort of healthy children. *Dev Med Child Neurol*. 2012;54(9):843-8.
- Turck D. Later effects of breastfeeding practice: the evidence. *Nestle Nutr Workshop Ser Pediatr Program*. 2007;60:31-9; discussion 39-42.

Vestergaard M, Obel C, Henriksen TB, Sørensen HT, Skajaa E, Ostergaard J. Duration of breastfeeding and developmental milestones during the latter half of infancy. *Acta Paediatr.* 1999;88(12):1327-32.

Wells JC, Jonsdottir OH, Hibberd PL, Fewtrell MS, Thorsdottir I, Eaton S, Lucas A, Gunnlaugsson G, Kleinman RE. Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: differences in breast-milk intake by stable-isotope probe. *Am J Clin Nutr.* 2012;96(1):73-9.

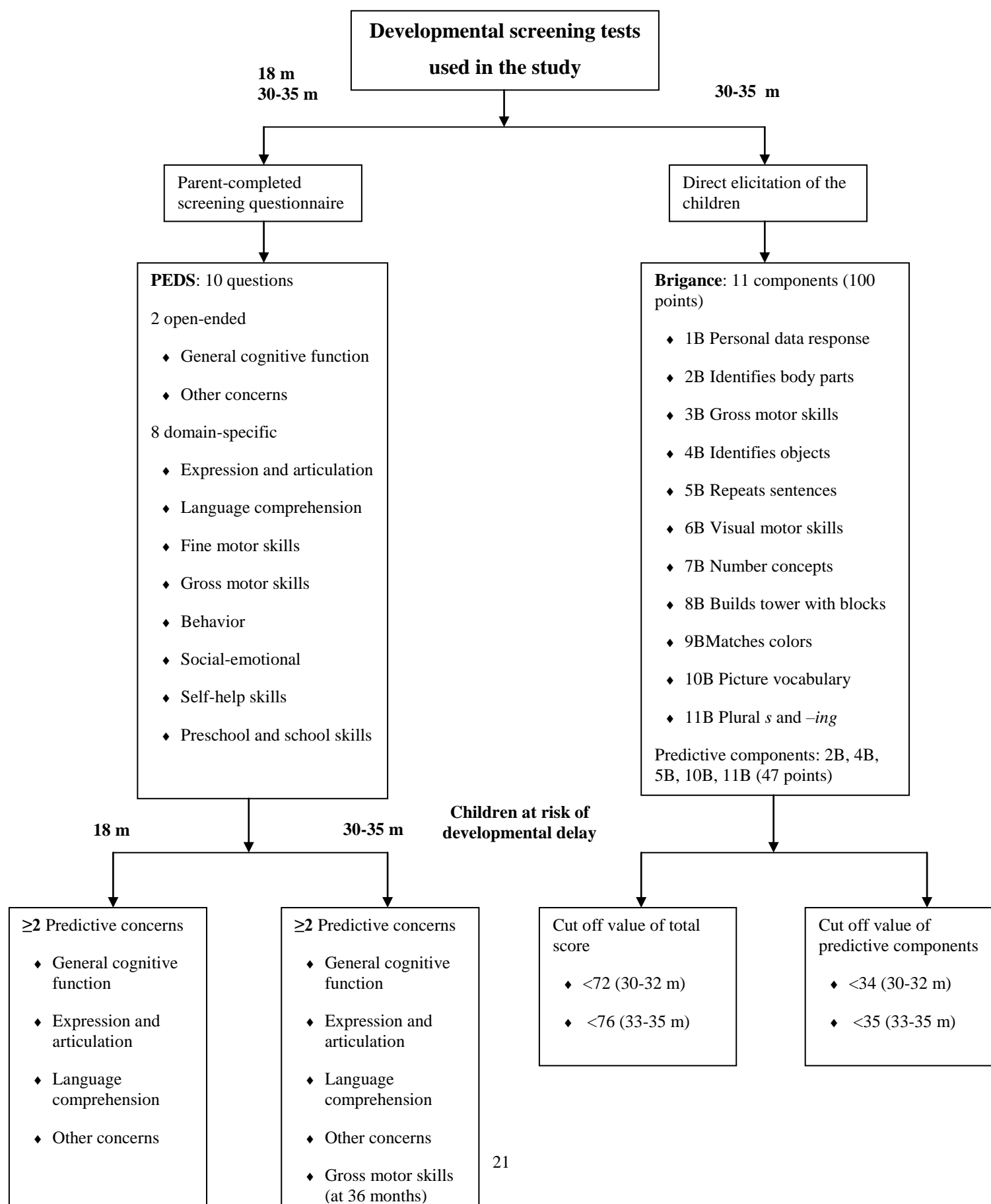
Whitehouse AJ, Robinson M, Li J, Oddy WH. Duration of breast feeding and language ability in middle childhood. *Paediatr Perinat Epidemiol.* 2011;25(1):44-52. doi: 10.1111/j.1365-3016.2010.01161.x

WHO. Indicators for assessing breastfeeding practices. Report of an informal meeting. Division of Child and Adolescent Health. WHO/CDD/SER/91.14.ed. Geneva: World Health Organization, 1991.

Wu TC, Chen PH. Health consequences of nutrition in childhood and early infancy. *Pediatr Neonatol.* 2009;50(4):135-42.

Zhou SJ, Baghurst P, Gibson RA, Makrides M. Home environment, not duration of breast-feeding, predicts intelligence quotient of children at four years. *Nutrition.* 2007;23(3):236-41.

## LEGENDS FOR ILLUSTRATIONS





**Figure 1.** Developmental screening tests used in the study, the PEDS questionnaire and the Brigance Screens-II.

**Table 1.** Baseline characteristics of participants with scores from Brigance Screens-II at 30-35 months of age in the two study groups: infants who received complementary foods in addition to breast milk from 4 months (CF, n=35) compared with infants who were exclusively breastfed for 6 months (EBF, n=31).

Variables	Group CF	Group EBF
Boys	17 (49%)*	13 (42%)*
Birth weight (g)	3687 (432)	3733 (526)
Length at birth (cm)	51.3 (1.8)	51.7 (1.9)
Head circumference at birth (cm)	35.8 (1.3) <sup>†</sup>	35.9 (1.4)
Gain in head circumference from birth - 18 months (cm)	12.6 (1.2) <sup>‡</sup>	12.6 (1.7) <sup>§</sup>
Age when Brigance Screens-II was performed (months)	32.3 (1.6)	32.8 (1.6)
Gestational length (days)	280.5 (9.3)	280.8 (7.1)
Maternal age (years)	29.4 (4.4)	31.2 (4.8)
Maternal education <sup>  </sup>	22 (63%)*	16 (52%)*
Vaginal delivery	33 (94%)*	23 (74%)*
Parity	2.0 (2.0) <sup>¶</sup>	2.0 (1.0) <sup>¶</sup>
Father's education <sup>  </sup>	13 (38%)* <sup>‡</sup>	14 (45%)*

Data are presented as mean (SD) unless otherwise indicated

\*Data are presented as number (%)

<sup>†</sup>One missing value, n=34

<sup>‡</sup>Three missing values, n=32

<sup>§</sup>Five missing values, n=26

<sup>||</sup>Finished studies at university level

<sup>¶</sup>Data are presented as median (IQR)

**Table 2.** Selected measures of developmental and behavioral status for children at 18 months (PEDS questionnaire: n=29 in group CF, n=25 in group EBF) and at 30–35 months of age (PEDS questionnaire: n=42 in group CF, n=36 in group EBF; Brigance Screens-II: n=35 in group CF, n=31 in group EBF) in the two intervention groups: infants who received complementary foods in addition to breast milk from 4 months (CF) compared with infants who were exclusively breastfed for 6 months (EBF).

Variables	Group CF	Group EBF	P-value
<i>PEDS questionnaire</i>			
Parents with concerns according to PEDS at 18 months	5 (17%)*	11 (44%)*	0.03
Parents with concerns according to PEDS at 30-35 months	14 (33%)*	15 (42%)*	0.45
<i>Brigance Screens-II</i>			
Total score at 30-35 months	86.0 (12.5) <sup>†</sup>	86.5 (12.5) <sup>†</sup>	0.82
Total score above cut off value <sup>‡</sup>	2 (6%)*	4 (13%)*	0.41
Score of predictive factors combined above cut off value <sup>§</sup>	7 (20%)*	3 (10%)* <sup>  </sup>	0.32
<i>Components of the Brigance Screens-II</i>			
Gross motor skills	6.0 (6.0) <sup>†</sup>	6.0 (4.5) <sup>†  </sup>	0.44
Fine motor skills	19.0 (3.0) <sup>†</sup>	19.0 (3.0) <sup>†  </sup>	0.89
Expressive and receptive language	40.5 (8.0) <sup>†</sup>	42.0 (9.5) <sup>†  </sup>	0.81

Data are presented as mean (SD) unless otherwise indicated

\*Data are presented as number (%)

<sup>†</sup>Data are presented as median (IQR)

<sup>‡</sup>Cut off values for defining risk of developmental delay were <72 and <76 points from the total score from the Brigance Screens-II for children aged 30-32 months and 33-35 months, respectively.

<sup>§</sup>Cut off values for defining risk of developmental delay were <34 and <35 points from the predictive components of the Brigance Screens-II combined for children aged 30-32 months and 33-35 months, respectively.

<sup>||</sup>Two missing values, n=29

# Paper IV

**Title:**

Exclusive breastfeeding for 4 versus 6 months and growth in early childhood

**Authors:**

Olof H Jonsdottir, BSc<sup>1</sup>, Ronald E Kleinman, MD<sup>2</sup>, Jonathan C Wells, PhD<sup>3</sup>, Mary S Fewtrell, MD<sup>3</sup>, Patricia L Hibberd, MD, PhD<sup>4</sup>, Geir Gunnlaugsson, MD, PhD<sup>5</sup>, Inga Thorsdottir, PhD<sup>1</sup>

**Affiliations:**

<sup>1</sup>Unit for Nutrition Research, Landspítali - The National University Hospital of Iceland and Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, Reykjavik, Iceland.

<sup>2</sup>Department of Pediatrics, Massachusetts General Hospital for Children, Harvard Medical School, Boston, MA, USA.

<sup>3</sup>Childhood Nutrition Research Centre, UCL Institute of Child Health, London, UK.

<sup>4</sup>Division of Global Health, Massachusetts General Hospital for Children, Harvard Medical School, Boston, USA.

<sup>5</sup>Directorate of Health and Reykjavik University, Iceland.

**Address correspondence to:**

Olof H Jonsdottir, Unit for Nutrition Research, Landspítali National University Hospital of Iceland, Eiríksgata 29; IS-101 Reykjavik, Iceland, Telephone: +354 543 1452, Fax: +354 543 4824, E-mail: [ohj@hi.is](mailto:ohj@hi.is)

**Short title:** Exclusive breastfeeding and growth in early childhood

**Abbreviations:** CF - Complementary feeding in addition to breast milk from the age of 4 months; EBF - Exclusive breastfeeding to the age of 6 months

**Key Words:** Exclusive breastfeeding, complementary feeding, randomized trial, growth

**Funding Source:** This study was supported by Mead Johnson and the Eimskip Fund of the University of Iceland. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, preparation of the report or the decision to submit for publication.

**Financial Disclosure:** The authors have no financial relationship relevant to this article to disclose.

**Conflict of Interest:** MF has received research funding from and undertaken advisory work for companies manufacturing infant foods and feeding products within the past 3 years, all the other authors declare that they have no conflicts of interests.

**Clinical Trial Registration:** ISRCTN41946519

**What's Known on This Subject:** Studies are inconsistent about whether duration of exclusive breastfeeding is protective of overweight and obesity later in life. Existing evidence on this relationship is based on observational studies with a risk of bias from confounding variables.

**What This Study Adds:** This randomized controlled trial showed no effects of exclusive breastfeeding for 4 or 6 months on the risk of being overweight or obese in early childhood.

**Contributor's Statement:**

Olof H Jonsdottir: Ms. Jonsdottir was responsible for the data collection and conducted the statistical analysis and wrote the first draft of the manuscript. Furthermore, she was responsible for the final decision to submit the manuscript.

Ronald E Kleinman: Dr. Kleinman was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Jonathan C Wells: Professor Wells was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Mary S Fewtrell: Dr. Fewtrell was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Patricia L Hibberd: Dr. Hibberd was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Geir Gunnlaugsson: Dr. Gunnlaugsson was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

Inga Thorsdottir: Professor Thorsdottir was involved in the structure and design of the present study, critically reviewed and revised the manuscript and approved the final manuscript for submission.

## ABSTRACT

**Objective:** To investigate the effect of duration of exclusive breastfeeding on weight gain and the risk of being overweight in later infancy and early childhood.

**Methods:** A total of 119 mother-infant pairs were randomized to either receive complementary foods from the age of 4 months in addition to breast milk (CF), or to be exclusively breastfed to 6 months (EBF). Each infant's weight, length and head circumference were measured at birth, 6 weeks, 3, 4, 5 and 6 months of age. In the follow-up the children's weight, length and head circumference were measured at 8, 10, 12 and 18 months and infant's weight and height at 29–38 months. All anthropometric measurements were converted to z scores using the WHO Child Growth Standards.

**Results:** A difference was seen between groups in gain in length from 4-18 months of age, where infants in the CF group ( $-0.17 \pm 0.84$ ) had significantly greater gain in length compared with the EBF group ( $-0.51 \pm 0.84$ ;  $p=0.05$ ). However, no difference was seen in the prevalence between groups in risk of being overweight or those who were overweight at 18 months and 29-38 months of age.

**Conclusions:** The differences in length seen between the two groups in later infancy to 18 months of age remain to be explained and may be a result of differences in dietary intake between groups. Exclusive breastfeeding for 4 or 6 months does not seem to affect risk of being overweight or the prevalence of those who were overweight or obese in early childhood.



## INTRODUCTION

Nutrition in early life is thought to influence the risk of overweight and obesity in childhood and adulthood, in part by affecting early growth patterns.<sup>(1)</sup> It has been suggested that more rapid infant weight gain, often seen after 4 months of age among formula fed infants, may lead to overweight or obesity in childhood in resource rich countries.<sup>(2-4)</sup> It has also been proposed that breastfeeding may be protective for overweight and obesity later in life, and this has been confirmed in several systematic reviews.<sup>(5-10)</sup>

Studies are inconsistent however about whether the total duration and exclusivity of breastfeeding affects the risk of being overweight or obese in childhood. In a very recent systematic review, it was concluded that exclusive breastfeeding for longer than 4 months may be associated with slower weight gain in late infancy. In addition, longer duration of exclusive breastfeeding or any breastfeeding may be protective against overweight and obesity in childhood and adolescence.<sup>(11)</sup> Others though suggest that duration or exclusivity of breastfeeding is not associated with the risk of being overweight or obese in early childhood.<sup>(12-16)</sup>

Most of existing evidence on the relationship between breastfeeding and weight gain in infancy and childhood is based on observational studies with significant risk of bias from confounding variables.<sup>(17-19)</sup> To minimize the effects of many of these confounding variables, we conducted a randomized controlled trial in Iceland to investigate both immediate and longer term health outcomes of exclusive breastfeeding for the first 4 vs. 6 months of life.<sup>(20,21)</sup> In this report we present the effects of exclusive breastfeeding for 4 vs. 6 months on growth in later infancy and early childhood. We hypothesized that a lower prevalence of overweight in early childhood would be seen among infants exclusively breastfed for 6

months compared with those receiving complementary foods from 4 months of age in addition to breast milk.

## **METHODS**

### **Study design**

A total of 119 mother-infant pairs were randomized between November 2007 and 2009 at 7 health care centers in Iceland, as previously reported.<sup>(20,21)</sup> Mothers of eligible infants (singleton birth, gestational length  $\geq 37$  weeks, exclusively breastfed, infant characterized as healthy: absence of congenital abnormalities or chronic health issues likely to affect growth, development or iron status) were informed about the study while attending well-baby clinic at their health care center when their infant was 2 months old. If the infant was still exclusively breastfed at 3 months the mothers were given more detailed information about the study with a study handout. Eligible mother-infant pairs were invited to participate in the study through phone calls and if the mother was interested in participating in the study, she and her infant were seen for a 4-month visit at their health care center. At this visit the mother-infant pairs were randomized either to receive complementary foods from the age of 4 months in addition to breast milk (CF), or to continue exclusive breastfeeding to the age of 6 months (EBF). Vitamin D supplements were recommended in both groups. Mothers in both groups were randomized by a nurse who also was an International Board Certified Lactation Consultant (IBCLC). All of the mothers also received lactation counseling from the nurse. Exclusive breastfeeding was defined as breastfeeding with no additional liquid or solid foods other than vitamins and medications.<sup>(22)</sup> The use of up to 10 feedings of formula or water during the first 6 months was allowed to avoid having to exclude infants that in fact were otherwise exclusively breastfed. 100 infants finished all measurements of the clinical trial. At

5 months postpartum the mothers were weighed at the health care center by the nurse or provided a self-reported weight.

The study was reviewed and approved by the Data Protection Authority and National Bioethical Committee in Iceland and the Partners Health System IRB.

### **Anthropometric measurements**

Each infant's weight, length and head circumference were measured at birth, 6 weeks, 3, 4, 5 and 6 months of age, as previously described.<sup>(21)</sup> Follow-up was done during routine scheduled visits at their health care centers where the infant's weight, length and head circumference are measured at 8, 10, 12 months and the child's weight and height at 18 and 29–38 months. Infants were consistently weighed completely unclothed on either a Seca model 727 or model 757 scale, (Vogel & Halke, Germany), depending on the health care center. Length was measured with the infant placed supine on a standard measuring board with a sliding foot plate (Seca model 207, Vogel & Halke, Germany) to 30 months of age. After 18 months of age, the children's weight was measured without shoes or socks while standing erect on a Seca model 761 scale (Vogel & Halke, Germany) and their height measured with Seca Body meter 208 (Vogel & Halke, Germany), depending on the health care center. Head circumference was measured with non-stretchable tape at all ages. Infants were measured once at each visit they made by an experienced child health nurse. All anthropometric measures were converted to z scores using the growth standards for children 0-59 months from the World Health Organization (WHO).<sup>(23)</sup> Cut points for classifying overweight and obesity in study subjects were according to the standards set forth by the WHO. Risk for overweight was defined as BMI-for-age >1 standard deviation (SD), above the WHO growth standard median. Overweight or obese was defined as a value >2 and >3 SDs, respectively, above the WHO growth standard median.<sup>(24)</sup>

## **Statistical analysis**

The statistical analysis was conducted using SPSS statistical software package version 20.0 (SPSS Inc., Chigaco, IL, USA). Data were presented as mean and SD for continuous normally distributed variables, as median and interquartile range (IQR) for continuous skewed variables and as counts and percentages for categorical variables. For comparison between the two study groups, the independent t-test or Mann-Whitney U-test were used for continuous variables and Chi-square tests of association or two-sided Fisher's exact test were used for categorical variables. Regression analysis was performed to test the impact of the intervention group and adjustment for baseline characteristics that were different at baseline. The level of significance was taken as  $p \leq 0.05$ . The power calculations were based on the primary outcome of the study, breast-milk intake. <sup>(20)</sup> However with at least 37 subjects per group, we had 80% power to detect a moderate effect size of 0.67 or greater using a 2 group t-test with a 2 sided significance level of 0.05.

## **RESULTS**

### **Participants and characteristics**

Of the 100 infants who completed the intervention trial a total of 97, 96, 98, 95 and 82 infants/children attended the routine care visit at their health care center at 8, 10, 12, 18 and 29-38 months, respectively. There was a missing data for anthropometric outcomes for 1 child who attended the routine care at 18 months and 5 children at 29-38 months and therefore data was available from 94 and 77 children at these ages. In an earlier reported study of this cohort we observed no statistical differences in baseline charactersitics between the ones who finished the intervention trial (n=100) and those who did not finish (n=19). <sup>(21)</sup> Of the baseline charactersitics, only the mode of delivery remained significantly different between the two

study groups at 29–38 months, where vaginal delivery was more common among subjects in group CF (90% vs. 68% in group EBF,  $p = 0.02$ ) (see **Table 1**). No significant differences were seen in the baseline characteristics presented in table 1 between those who were followed-up ( $n=82$ ) and those lost to follow-up ( $n=18$ ), except for parity, where those who were lost to follow-up had significantly more children ( $3.0\pm1.0$  children for those not followed-up vs.  $2.0\pm2.0$  children for those who were followed-up;  $p=0.01$ ).

### **Anthropometric outcomes**

No differences were seen between study groups in mean z scores for age for weight, length/height, head circumference or BMI from birth to 4 months of age, prior to randomization. **Figure 1** shows the change in height-for-age and head circumference-for-age according to mean z scores from birth to 29-38 months of age. From the start of the intervention at 4 months of age, up to 18 months of age the gain in length was significantly greater among infants in the CF group ( $0.17\pm0.84$ ,  $n=48$ ) compared with infants in the EBF group ( $-0.51\pm0.84$ ,  $n=46$ ;  $p=0.05$ ). Faster gain in BMI between 10-12 months was also seen among infants in the CF group ( $0.27\pm0.54$ ,  $n=45$ ) compared with those in the EBF group ( $0.06\pm0.47$ ,  $n=48$ ;  $p=0.05$ ). Furthermore, infants in the CF group had faster weight gain from 10-18 months ( $0.32\pm0.48$ ,  $n=46$ ) compared with those in the EBF group ( $0.11\pm0.57$ ,  $n=46$ ), although this was of borderline significance ( $p=0.06$ ) (see **Figure 2**). Since mode of delivery was significantly different between groups at baseline, a linear regression analysis was performed to test the impact of the intervention group on growth. Length gain from 4–18 months was still significantly different between study groups when adjusted for mode of delivery ( $p=0.04$ ) and also gain in BMI from 10–12 months ( $p=0.04$ ) and the weight gain from 10–18 months ( $p=0.035$ ).

**Table 2** compares the number of children at risk of being overweight ( $>1$  SD), those who were overweight ( $>2$  SDs) or obese ( $>3$  SDs) at 18 months and 29–38 months according to z scores of BMI-for-age between the two study groups. There was neither a significant difference between the two study groups in number of children at risk of being overweight nor those who were overweight at 18 months and 29–38 months of age. None of the children in the present study were obese at these ages according to the WHO growth standards. The mean BMI z score-for-age for children was  $0.60 \pm 0.92$  and  $0.59 \pm 0.95$  at 18 months for children in group CF and EBF, respectively ( $p=0.96$ ) and  $0.64 \pm 0.86$  and  $0.79 \pm 0.83$  at 29–38 months of age for children in the CF and EBF group, respectively ( $p=0.45$ ). At 18 months of age, 29% of infants in the CF group and 33% in the EBF group were at risk of being overweight ( $p=0.72$ ) and 8% in the CF group and 11% in the EBF group were overweight ( $p=0.74$ ). At 29–38 months of age, 30% of infants in the CF group were at risk of being overweight compared with 38% of those in the EBF group ( $p=0.47$ ) and 10% of the CF group compared with 3% of the EBF group were overweight ( $p=0.36$ ). Of those infants in the EBF group, 7 mothers gave them formula before 6 months, most of them only once. There was no change in the prevalence of overweight seen among groups by excluding these infants.

## DISCUSSION

This randomized controlled trial of the effects of exclusive breastfeeding for 4 vs. 6 months, showed a difference in length between the two study groups between 4–18 months of age, where subjects in the CF group grew significantly faster than those in the EBF group. No difference was seen in growth from birth to 4 months, indicating that the intervention from 4–6 months may have affected the growth rate up to 18 months of age but this effect was not detectable at the age of 29–38 months. No difference was seen between study groups in the

percentage of children at risk of being overweight or those who were overweight at 18 months and 29-38 months.

Rapid weight gain in infancy is thought to be a risk factor for overweight and obesity in childhood and later in life in resource rich countries. <sup>(2,3,25)</sup> Studies investigating nutrition in infancy and the risk of being overweight or obese most often compare breastfeeding and formula feeding. <sup>(26)</sup> Less is known however about the effect of the duration of exclusive breastfeeding on the risk of overweight and obesity in childhood. A recently reported cross-sectional study from 8 European countries showed that exclusive breastfeeding for 6 months was more protective against childhood overweight and obesity than exclusive breastfeeding for 4 and 5 months. <sup>(27)</sup> However, overall, studies are inconsistent about whether duration of exclusive breastfeeding affects the risk of being overweight or obese in childhood and some studies show no relationship between exclusive breastfeeding duration and later risk of obesity. A randomized controlled trial, conducted in a resource poor country, showed no difference in weight gain up to 12 months of age between infants exclusively breastfed for 4 months compared with those exclusively breastfed for 6 months. <sup>(28)</sup> Furthermore, a large cluster-randomized trial of a breastfeeding promotion in Belarus showed no association between mean BMI and the prevalence of obesity at 6.5 years of age with increased total duration and exclusivity of breastfeeding. <sup>(16)</sup>

It has been suggested that the more rapid weight gain in infancy and childhood that has been reported in formula fed infants after 4 months of age may partly be due to the higher protein intakes associated with formula feeding. <sup>(4, 29,30)</sup> A longer duration of total and exclusive breastfeeding, and therefore lower protein intake, could therefore be protective against later overweight and obesity. <sup>(31-35)</sup> Thus a possible reason for the faster length gain seen in the current study among children in the CF group after 4 months may be higher

protein intakes received from complementary foods between 4 and 6 months of age compared with protein intakes received solely from breast milk in the EBF group. However, this does not explain the persistent differences in length seen to 18 months of age. Unfortunately dietary intake data are not available for these study groups beyond the 4-6 months intervention period.

Breastfeeding on demand and later introduction of complementary foods may have beneficial effects on self-regulation of energy intake and feeding patterns later in childhood.<sup>(36, 37)</sup> In contrast, formula feeding on schedule in infancy could increase the risk of overweight in childhood due to less effective self-regulation of energy intake.<sup>(31)</sup> In this study, all infants were exclusively breastfed on demand for the first 4 months of life and thereafter breastfed until its termination. Therefore the theoretical relationship between breastfeeding and development of dietary self-regulation is unlikely to explain the observed difference in length gain between the two intervention groups. It is possible that the difference in gain in length in late infancy may be explained by differences in parental height. We were unable to exclude this as data were not available, although such differences should not be an issue in this randomized trial. The earlier introduction of complementary foods in the CF group did not appear to affect the risk of later overweight or obesity at 18 and 29-38 months of age in the current study.

Rapid postnatal growth in early life is often seen among low birth weight infants and those born small for gestational age (SGA). However, rapid growth in infancy has also been seen among infants who have an appropriate birth weight for their gestational age (AGA)<sup>(38,39)</sup> The mean weight of the infants in the present study were within 2 z scores of the WHO reference standards at birth and therefore they presumably experienced no prenatal growth restriction. All infants exclusively breastfed during the first 4 months of life and experienced



similar growth and none demonstrated significant growth acceleration or “catch-up” growth beyond the norm.

The World Health Organization WHO recommended exclusive breastfeeding for 4-6 months after birth until the year 2001 when the recommendations were changed to exclusive breastfeeding for the first 6 months of infants life. The principal strength of the current study is its randomized controlled trial design and to our knowledge this is the only randomized controlled trial, conducted in a resource rich country, investigating the effects of exclusive breastfeeding for 4 vs. 6 months on growth in early childhood. An additional strength of this study is the high percentage of children followed until the age of 29-38 months, approximately 82%. The relatively small sample size and the lack of information on father's weights is a limitation of the study. The findings support other intervention studies that show no effect of a longer duration of exclusive breastfeeding on overweight or obesity in early childhood.

## **CONCLUSION**

Exclusive breastfeeding for 4 or 6 months does not seem to affect risk of being overweight or the prevalence of those who were overweight or obese in early childhood. The differences in weight and length seen between those introduced to complementary foods at 4 months of age during later infancy to 18 months of age remain to be explained and may be a result of differences in dietary intake between groups.

## **ACKNOWLEDGEMENTS**

We are very grateful to all children and their parents who participated in the study.

Furthermore we greatly acknowledge the nurses Sesselja Gudmundsdottir, Dagny

Gudmundsdottir, Audur Egilsdottir and Kristin J. Vigfusdottir and the nurses and the lactation

consultants Jona Margret Jonsdottir, Alma Maria Rognvaldsdottir, Elin Sigurbjornsdottir,

Gudrun Gudmundsdottir and Ingibjorg Eiriksdottir for recruiting mother-infant pairs in the

study and their great amount of assistance in data collection.

## REFERENCES

1. Lucas A. Role of nutritional programming in determining adult morbidity. *Arch Dis Child*. 1994 Oct;71(4):288-90.
2. Corvalán C, Kain J, Weisstaub G, Uauy R. Impact of growth patterns and early diet on obesity and cardiovascular risk factors in young children from developing countries. *Proc Nutr Soc*. 2009;68(3):327-37.
3. Lamb MM, Dabelea D, Yin X, Ogden LG, Klingensmith GJ, Rewers M, Norris JM. Early-life predictors of higher body mass index in healthy children. *Ann Nutr Metab*. 2010;56(1):16-22.
4. Mahrshahi S, Battistutta D, Magarey A, Daniels LA. Determinants of rapid weight gain during infancy: baseline results from the NOURISH randomised controlled trial. *BMC Pediatr*. 2011;7(11):99.
5. Horta BL, Bahl R, Martinés JC, Victora CG. Evidence on the long-term effects of breastfeeding: Systematic reviews and meta-analyses. Geneva, Switzerland: World Health Organization; 2007.
6. Schack-Nielsen L, Michaelsen KF. Breast feeding and future health. *Curr Opin Clin Nutr Metab Care*. 2006;9(3):289-96.
7. Butte NF. Impact of infant feeding practices on childhood obesity. *J Nutr*. 2009;139(2):412S-6S.
8. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T, Lau J. Breastfeeding and maternal and infant health outcomes in developed countries. *Evid Rep Technol Assess (Full Rep)*. 2007;(153):1-186.
9. Schack-Nielsen L, Michaelsen KF. Advances in our understanding of the biology of human milk and its effects on the offspring. *J Nutr*. 2007;137(2):503S-510S.
10. Arenz S, Ruckerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity--a systematic review. *Int J Obes Relat Metab Disord*. 2004;28(10):1247-56.
11. Hörnell A, Lagström H, Lande B, Thorsdottir I. Breastfeeding, introduction of other foods and effects on health: A systematic literature review for the 5th Nordic Nutrition Recommendations. Food and Nutrition Research. [In press].
12. Durmuş B, van Rossem L, Duijts L, Arends LR, Raat H, Moll HA, Hofman A, Steegers EA, Jaddoe VW. Breast-feeding and growth in children until the age of 3 years: the Generation R Study. *Br J Nutr*. 2011;105(11):1704-11.

13. Balaban G, Motta ME, Silva GA. Early weaning and other potential risk factors for overweight among preschool children. *Clinics (Sao Paulo)*. 2010;65(2):181-7.
14. Al-Qaoud N, Prakash P. 'Can breastfeeding and its duration determine the overweight status of Kuwaiti children at the age of 3-6 years?' *Eur J Clin Nutr*. 2009;63(8):1041-3.
15. Novaes JF, Lamounier JA, Colosimo EA, Franceschini SC, Priore SE. Breastfeeding and obesity in Brazilian children. *Eur J Public Health*. 2012;22(3):383-9.
16. Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet JP, Martin RM, Davey Smith G, Gillman MW, Chalmers B, Hodnett E, Shapiro S; PROBIT Study Group. Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity, and blood pressure at age 6.5 y: evidence from a large randomized trial. *Am J Clin Nutr*. 2007;86(6):1717-21.
17. Owen CG, Martin RM, Whincup PH, Davey-Smith G, Gillman MW, Cook DG. The effect of breastfeeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. *Am J Clin Nutr*. 2005;82(6):1298-307.
18. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. *Pediatrics*. 2005;115(5):1367-77.
19. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev*. 2012;8:CD003517.
20. Wells JC, Jonsdottir OH, Hibberd PL, Fewtrell MS, Thorsdottir I, Eaton S, Lucas A, Gunnlaugsson G, Kleinman RE. Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: differences in breast-milk intake by stable-isotope probe. *Am J Clin Nutr*. 2012;96(1):73-9.
21. Jonsdottir OH, Thorsdottir I, Hibberd PL, Fewtrell MS, Wells JC, Palsson GI, Lucas A, Gunnlaugsson G, Kleinman RE. Timing of the introduction of complementary foods in infancy: A randomized controlled trial. *Pediatrics*. 2012;130(6):1038-45.
22. WHO. Indicators for assessing breastfeeding practices. Report of an informal meeting. Division of Child and Adolescent Health. WHO/CDD/SER/91.14.ed. Geneva: World Health Organization, 1991.
23. WHO Anthro for personal computers, version 3.1, 2010: Software for assessing growth and development of the world's children. Geneva, Switzerland: WHO; 2010. Available at <http://www.who.int/childgrowth/software/en/>.
24. World Health Organization. Training course on child growth assessment. Geneva, Switzerland: WHO; 2008. Available at [http://www.who.int/childgrowth/training/module\\_c\\_interpreting\\_indicators.pdf](http://www.who.int/childgrowth/training/module_c_interpreting_indicators.pdf). Accessed October 22<sup>nd</sup>, 2012.
25. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. *BMJ*. 2005;331(7522):929.
26. Weng SF, Redsell SA, Swift JA, Yang M, Glazebrook CP. Systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy. *Arch Dis Child*. 2012 Oct 29. [Epub ahead of print]
27. Hunsberger M, Lanfer A, Reeske A, Veidebaum T, Russo P, Hadjigeorgiou C, Moreno LA, Molnar D, De Henauw S, Lissner L, Eiben G. Infant feeding practices and prevalence of obesity in eight European countries - the IDEFICS study. *Public Health Nutr*. 2012 Aug 24:1-9.

28. Dewey KG, Cohen RJ, Brown KH, Rivera LL. Age of introduction of complementary foods and growth of term, low-birth-weight, breast-fed infants: a randomized intervention study in Honduras. *Am J Clin Nutr.* 1999;69(4):679-86.
29. Koletzko B, von Kries R, Closa R, Escibano J, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Gruszfeld D, Dobrzanska A, Sengier A, Langhendries JP, Rolland Cachera MF, Grote V; European Childhood Obesity Trial Study Group. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. *Am J Clin Nutr.* 2009;89(6):1836-45.
30. Escibano J, Luque V, Ferre N, Mendez-Riera G, Koletzko B, Grote V, Demmelmair H, Bluck L, Wright A, Closa-Monasterolo R; European Childhood Obesity Trial Study Group. Effect of protein intake and weight gain velocity on body fat mass at 6 months of age: the EU Childhood Obesity Programme. *Int J Obes (Lond).* 2012;36(4):548-53.
31. Griffiths LJ, Smeeth L, Hawkins SS, Cole TJ, Dezateux C. Effects of infant feeding practice on weight gain from birth to 3 years. *Arch Dis Child.* 2009;94(8):577-82.
32. Gillman MW, Rifas-Shiman SL, Camargo CA Jr, Berkey CS, Frazier AL, Rockett HR, Field AE, Colditz GA. Risk of overweight among adolescents who were breastfed as infants. *JAMA.* 2001;285(19):2461-7.
33. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. Breast feeding and obesity: cross sectional study. *BMJ.* 1999;319(7203):147-50.
34. Gopinath B, Subramanian I, Flood VM, Baur LA, Pfund N, Burlutsky G, Mitchell P. Relationship between breast-feeding and adiposity in infants and pre-school children. *Public Health Nutr.* 2012; 28:1-6.
35. Gunnarsdottir I, Schack-Nielsen L, Michaelsen KF, Sørensen TI, Thorsdottir I; NordNet Study Group. Infant weight gain, duration of exclusive breast-feeding and childhood BMI - two similar follow-up cohorts. *Public Health Nutr.* 2010;13(2):201-7.
36. Fisher JO, Birch LL, Smiciklas-Wright H, Picciano MF. Breast-feeding through the first year predicts maternal control in feeding and subsequent toddler energy intakes. *J Am Diet Assoc.* 2000;100(6):641-6.
37. Abraham EC, Godwin J, Sherriff A, Armstrong J. Infant feeding in relation to eating patterns in the second year of life and weight status in the fourth year. *Public Health Nutr.* 2012;1-10. [Epub ahead of print]
38. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ.* 2000;320(7240):967-71.
39. Karaolis-Danckert N, Buyken AE, Kulig M, Kroke A, Forster J, Kamin W, Schuster A, Hornberg C, Keil T, Bergmann RL, Wahn U, Lau S. How pre- and postnatal risk factors modify the effect of rapid weight gain in infancy and early childhood on subsequent fat mass development: results from the Multicenter Allergy Study 90. *Am J Clin Nutr.* 2008;87(5):1356-64.

## LEGENDS OF ILLUSTRATION

**Table 1.** Baseline characteristics of the participants with anthropometric measurements at 29 - 38 months of age in the two intervention groups: infants who received complementay foods from 4 months of age in addition to breast milk (CF, n=40) compared with infants who were exclusively breastfed for 6 months (EBF, n=37).

Variables	Group CF	Group EBF	P-value
Boys	20 (50%)*	16 (43%)*	0.55
Birth weight (g)	3692 (448)	3720 (573)	0.81
Birth weight (z score)	0.80 (0.89)	0.84 (1.09)	0.86
Length at birth (cm)	51.4 (1.8)	51.7 (2.1)	0.52
Length at birth (z score)	1.01 (0.95)	1.19 (1.04)	0.43
Head circumference at birth (cm)	35.7 (1.2) <sup>†</sup>	35.9 (1.4)	0.53
Head circumference at birth (z score)	1.30 (1.04) <sup>†</sup>	1.46 (1.01)	0.49
Vaginal delivery	36 (90%)*	25 (68%)*	0.02
Maternal age (years)	28.9 (4.2)	30.7 (5.3)	0.12
Maternal education <sup>‡</sup>	24 (60%)*	18 (49%)*	0.32
Father's education <sup>‡</sup>	13 (33%)* <sup>†</sup>	14 (38%)*	0.68
Gestational length (days)	281.4 (9.6)	280.0 (7.7)	0.47
Parity	2.0 (2.0) <sup>§</sup>	2.0 (2.0) <sup>§</sup>	0.43
Maternal weight 5 months postpartum (kg)	73.9 (16.3)	71.8 (12.2) <sup>  </sup>	0.55

Values are mean (SD) unless otherwise indicated

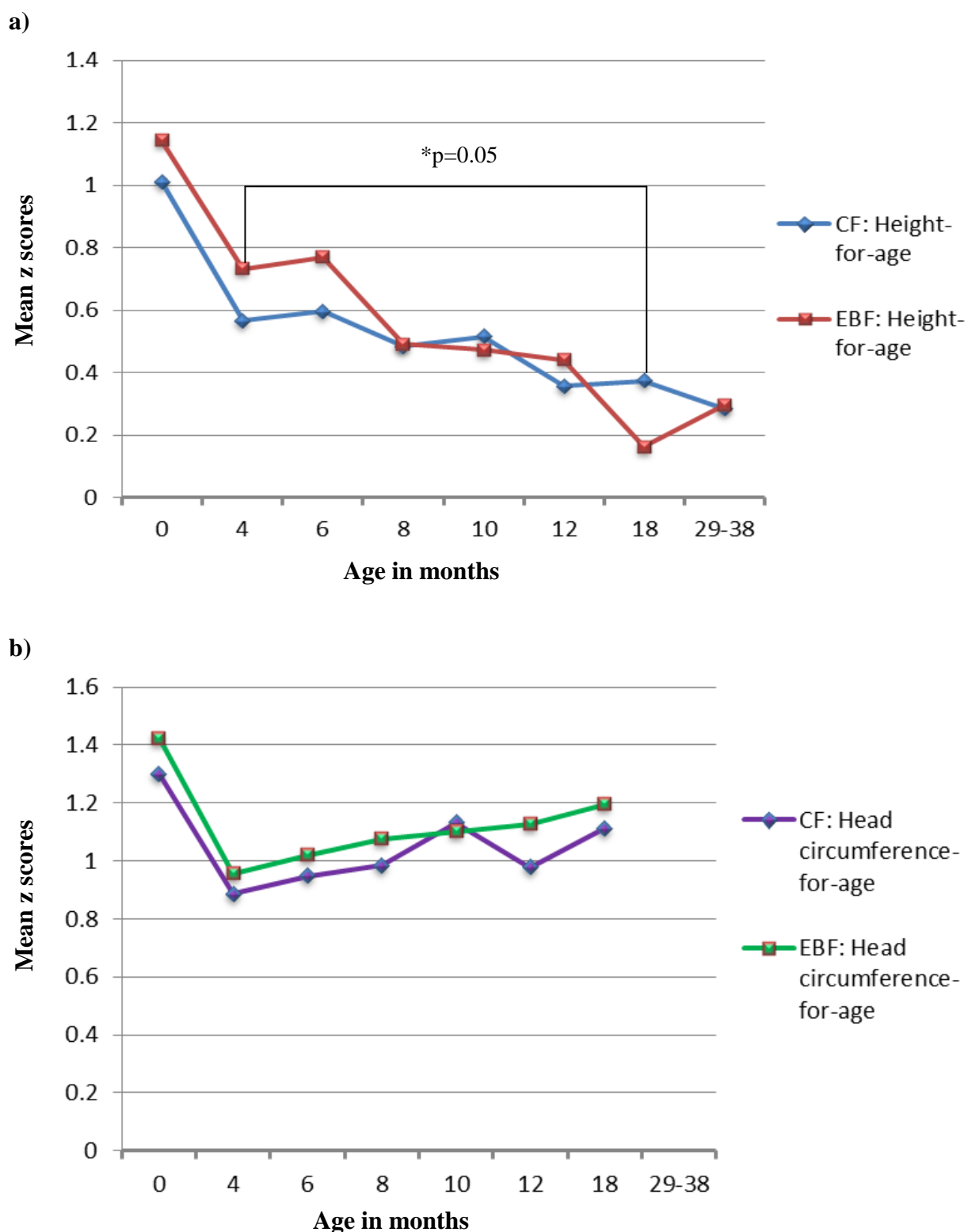
\*Data are presented as number (%)

<sup>†</sup>One missing value, n=39

<sup>‡</sup>Finished studies at university level

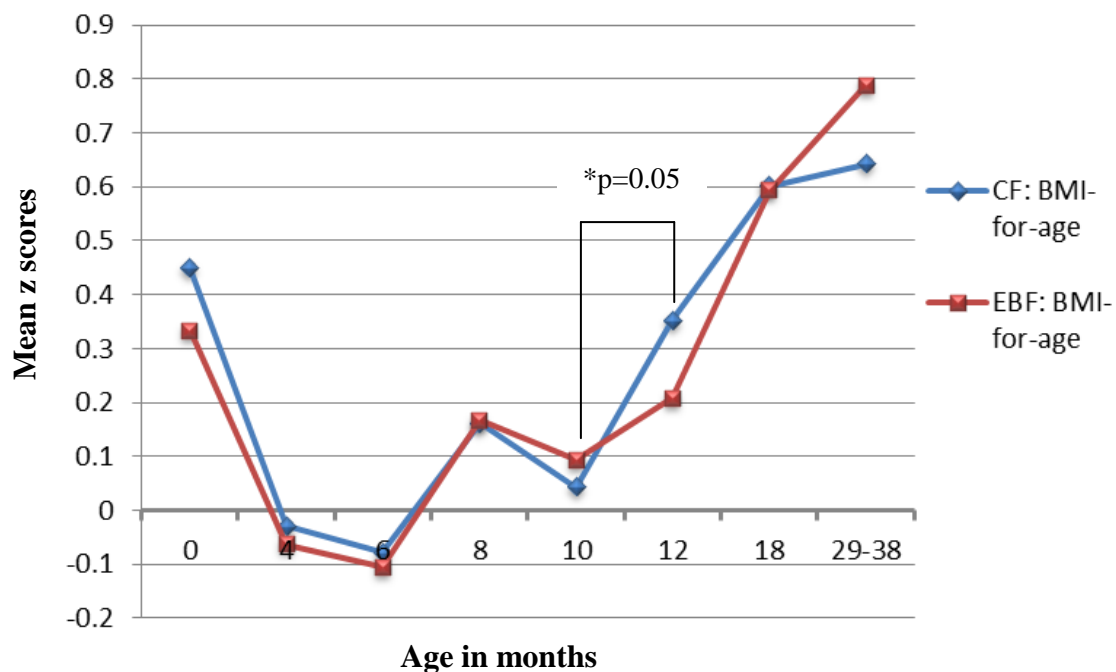
<sup>§</sup>Data are presented as median (IQR)

<sup>||</sup>Two missing values, n=35

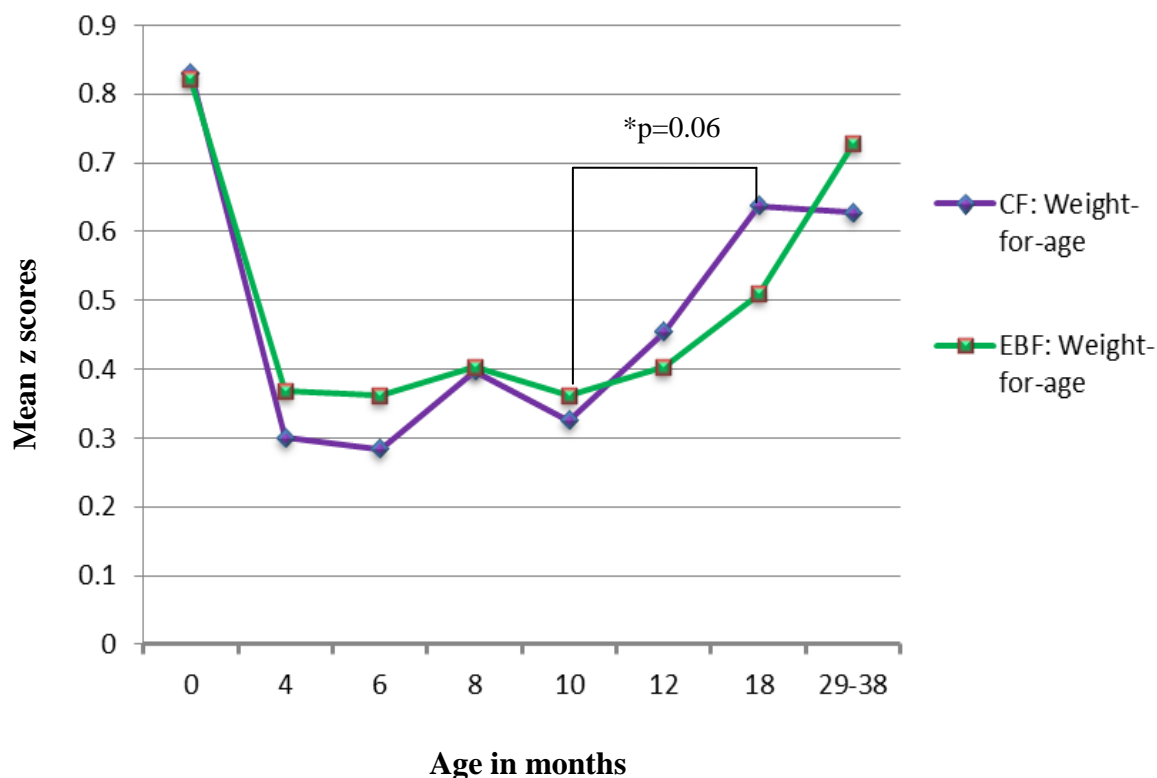


**Figure 1.** Mean height-for-age (a) and mean head circumference-for-age (b) from birth to 29-38 months of age calculated in z scores in the two study groups: infants who received complementay foods from 4 months of age in addition to breast milk (CF) compared with infants who were exclusively breastfed for 6 months (EBF).

a)



b)



**Figure 2.** Mean BMI-for-age (a) and mean weight-for-age (b) from birth to 29-38 months of age calculated in z scores in the two study groups: infants who received complementay foods from 4 months of age in addition to breast milk (CF) compared with infants who were exclusively breastfed for 6 months (EBF).

**Table 2.** BMI-for-age in z scores\* and the prevalence of children at risk of overweight (>1 SD), were overweight (>2 SD) or obese (>3 SD) at 18 months and 29 - 38 months of age in the two intervention groups: infants who received complementay foods from 4 months of age in addition to breast milk (CF; n=48 at 18 months, n= 40 at 29-38 months) compared with infants who were exclusively breastfed for 6 months (EBF; n=46 at 18 monts, n= 37 at 29-38 months).

Variables	Group CF	Group EBF	p-value
<i>18 months of age</i>			
BMI-for-age	0.60±0.92	0.59±0.95	0.96
Risk of overweight	14 (29%) <sup>†</sup>	15 (33%) <sup>†</sup>	0.72
Overweight	4 (8%) <sup>†</sup>	5 (11%) <sup>†</sup>	0.74
Obese	0	0	-
<i>29-38 months of age</i>			
BMI-for-age	0.64±0.86	0.79±0.83	0.45
Risk of overweight	12 (30%) <sup>†</sup>	14 (38%) <sup>†</sup>	0.47
Overweight	4 (10%) <sup>†</sup>	1 (3%) <sup>†</sup>	0.36
Obese	0	0	-

Data are presented as mean (SD) unless otherwise indicated

\* z-scores calculated using the WHO infant growth standards

<sup>†</sup>Number (%)



# Paper V

**Title:**

Initiation of complementary feeding and duration of total breastfeeding: unlimited access to lactation consultants *versus* routine care at the well-baby clinics.

**Authors:**

Olof H Jonsdottir<sup>1</sup>, Mary S Fewtrell<sup>2</sup>, Geir Gunnlaugsson<sup>3</sup>, Ronald E Kleinman<sup>4</sup>, Patricia L Hibberd<sup>5</sup>, Jona M Jonsdottir<sup>6</sup>, Ingibjorg Eiriksdottir<sup>6</sup>, Alma M Rognvaldsdottir<sup>6</sup>, Inga Thorsdottir<sup>1</sup>

**Affiliations:**

<sup>1</sup>Unit for Nutrition Research, Landspítali - The National University Hospital of Iceland and Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, Reykjavik, Iceland.

E-mails: [ohj@hi.is](mailto:ohj@hi.is), [ingathor@hi.is](mailto:ingathor@hi.is)

<sup>2</sup>Childhood Nutrition Research Centre, UCL Institute of Child Health, London, UK.

E-mail: [m.fewtrell@ucl.ac.uk](mailto:m.fewtrell@ucl.ac.uk)

<sup>3</sup>Directorate of Health and Reykjavik University, Iceland.

E-mail: [geirg@hr.is](mailto:geirg@hr.is)

<sup>4</sup>Department of Pediatrics, Massachusetts General Hospital for Children, Harvard Medical School, Boston, MA, USA.

E-mail: [rkleinman@partners.org](mailto:rkleinman@partners.org)

<sup>5</sup>Division of Global Health, Massachusetts General Hospital for Children, Harvard Medical School, Boston, USA.

E-mail: [phibberd@partners.org](mailto:phibberd@partners.org)

<sup>6</sup>Primary Health Care of the Capital Area, Reykjavik, Iceland.

E-mails: [jona.margret.jonsdottir@heilsugaeslan.is](mailto:jona.margret.jonsdottir@heilsugaeslan.is) , [ingibjorg.eiriksdottir@heilsugaeslan.is](mailto:ingibjorg.eiriksdottir@heilsugaeslan.is) , [alma.m.rognvaldsdottir@heilsugaeslan.is](mailto:alma.m.rognvaldsdottir@heilsugaeslan.is)

**Corresponding author:**

Olof H Jonsdottir

Unit for Nutrition Research,

Landspítali National University Hospital of Iceland, Eiríksgata 29; IS-101 Reykjavik, Iceland

Telephone: +354 543 1452

Fax: +354 543 4824

E-mail: [ohj@hi.is](mailto:ohj@hi.is)

## ABSTRACT

**Background:** Breastfeeding has a number of advantages for both mother and child. Lactation consultants may promote prolonged breastfeeding but little is known about their impact on the initiation of complementary feeding. The aim of the study was to investigate the types of complementary foods and number of meals introduced from 5-6 months of age by mothers who had either unlimited access to lactation consultants or received routine care at the well-baby clinics at the primary health care centers. We also studied the duration of total breastfeeding.

**Methods:** Dietary intake during the initial complementary feeding period from 5 to 6 months was collected on mother-infant pairs that had unlimited access to lactation consultants along with those mother-infant pairs that received routine care at the well-baby clinics. The total duration of breastfeeding in each study population was also recorded and comparison performed of total breastfeeding durations of infants receiving complementary foods from 4 months and those exclusively breastfed for 6 months in each of the two study populations.

**Results:** Higher proportion of infants of mothers with unlimited access to lactation consultants were fed vegetable and vegetable purées ( $p=0.05$ ) and more than one food type ( $p=0.05$ ) at 5 months. Furthermore, a lower percentage of them had three meals per day at 6 months ( $p=0.001$ ) compared with those receiving routine care at the well-baby clinics. Infants exclusively breastfed for 6 months all had similar duration of total breastfeeding. Those of mothers who had unlimited access to lactation consultants had longer duration of total breastfeeding ( $1.08\pm0.15$ ) than infants receiving complementary foods from 4 months of age, whose mothers also had unlimited access to lactation consultant ( $1.01\pm0.11$ , 95% CI -0.001, 0.16) and whose mothers had routine care at the well-baby clinics ( $0.96\pm0.13$ , 95% CI 0.03, 0.22).

**Conclusions:** Mother-infant pairs with unlimited access to lactation consultant had slower introduction of complementary foods at the initial complementary feeding period, according to number of infant's meals at 6 months of age. Furthermore, those exclusively breastfed for 6 months had more prolonged breastfeeding compared with mothers who begin complementary feeding at 4 months regardless of exposure to lactation consultants.

**Keywords:** Lactation consultant, exclusive breastfeeding, complementary feeding, total breastfeeding duration.

**Trial registry number of the randomized controlled trial:** ISRCTN 41946519

## **BACKGROUND**

Nutrition at early developmental stages in life seems to have long-term effects on health outcomes later in life and the risk of non-communicable diseases. The mechanism is thought to be through nutritional influences on growth.<sup>[1,2]</sup> Studies indicate that rapid growth in infancy is associated with increased risk of childhood obesity<sup>[3,4]</sup> and that prolonged breastfeeding may have protective effects on overweight and obesity in childhood and adolescence.<sup>[5-7]</sup>

Breastfeeding support is critical to breastfeeding success. Pre- and post-natal education and support by lactation consultants is strongly associated with a longer duration of breastfeeding<sup>[8-13]</sup> A recent systematic review showed that professional breastfeeding support at the individual level increased the rate of breastfeeding between 4 and 5 months of age, a time when breastfeeding rates typically decline sharply.<sup>[14]</sup> Very little is known about the impact of lactation consultants on the process of complementary feeding, including the frequency of giving complementary foods and the choice of foods provided to infants early in the complementary feeding process. Complementary feeding at this critical time period in early life might be influenced by lactation consultants and furthermore the complementary foods might influence growth in infancy and therefore future health outcomes.<sup>[15]</sup>

The aim of the present study was to assess the effect of unlimited access to lactation consultants on complementary feeding from 5-6 months for infants receiving complementary foods from 4 months of age in addition to breast milk and their effect on total breastfeeding duration.

## **METHODS**

## **Participants and study design**

In this study we examined complementary feeding practices between 5 to 6 months of age and total breastfeeding duration by comparing dietary intake data from study subjects from two previously reported studies.<sup>[16,17,18]</sup> One of the two studies was a randomized controlled trial where participating mothers received counseling from an International Board Certified Lactation Consultant and had unlimited access to the lactation consultants. The other study was a national prospective cohort study where mothers and infants received routine care at the well-baby clinics at the primary health care centers. Both studies from which the current analysis was conducted had similar eligibility criteria and were conducted at a similar time period, thus providing the opportunity to compare the complementary feeding practices during the initial complementary feeding period and total duration of breastfeeding, based on exposure to professional lactation consultation.

The sample of mother-infant pairs that had unlimited access to lactation consultants was recruited in 7 health care centers in Iceland between November 2007 and November 2009 into a randomized controlled trial.<sup>[16,17]</sup> Infants were randomly assigned either to receive complementary foods from the age of 4 months in addition to breast milk (n = 50) or to continue being exclusively breastfed to the age of 6 months (n = 50). Randomization was performed, as previously described,<sup>[16,17]</sup> by a lactation consultant at their health care center when the infant was 4 months of age. During the study the mothers could at all times contact them for support and advice.

The sample of mother-infant pairs receiving routine care at the well-baby clinics was from a national prospective cohort study of infants born between January and December 2005 that were randomly selected by Statistics Iceland when the infants were 4 months old. During

the study the infants received routine care at the well-baby clinics.<sup>[18]</sup> A total of 250 infants were collected in the study and thereof 28 infants received complementary foods from 4 months of age in addition to breast milk and 15 infants were exclusively breastfed for 6 months. These 43 infants were included in the current study.

The infants selected in both studies were born in Iceland and fulfilled similar inclusion criteria for participation, i.e. singleton birth, gestational length at least 37 weeks and absence of congenital abnormalities or chronic health issues. Exclusive breastfeeding was defined as breastfeeding with no additional liquid or solid foods other than vitamin drops and medications,<sup>[19]</sup> although infants of mothers having unlimited access to lactation consultants were allowed to receive up to 10 feedings of formula or water during the first 6 months. Complementary feeding was defined as consumption of any solid, semi-solid foods or liquid with continued breastfeeding. Meals were only included in the comparison of number of meals if the infant had solid, semi-solid or soft foods, formula feeding alone did not count as a meal. The primary outcome in the current study was a comparison of the number of food types at 5 months and number of meals between the two study populations at 5-6 months of age. The secondary outcome was a comparison of total breastfeeding duration among the infants.

Both studies were reviewed and approved by the Data Protection Authority and National Bioethical Committee in Iceland and the randomized controlled trial was also approved by the Partners Health System IRB, Boston, MA.

### **Advice on complementary feeding**

Preventive health services for 0-5 year old children in Iceland are based on guidelines, published by the Directorate of Health.<sup>[20]</sup> In line with those guidelines, infants in both

cohorts came for regular visits at their health care center at 6 and 9 weeks, 3, 5 and 6 months of age and met a nurse in all visits and a doctor at 3 and 6 months. A booklet, the infant dietary recommendations <sup>[21]</sup> was provided to all mothers in both cohorts during one of these visits to the health care center, and the parents advised about complementary feeding. Mothers receiving routine care at the well-baby clinics received recommendations about complementary foods in the visit to the health care centers at 5 months if the nurse judged it important to educate the mother about complementary foods or if the mother asked for advice about the complementary feeding. At the same visit the infant's weight, length and head circumference was measured and they also received immunizations. The mothers were advised to give their infants porridges and purées of fruits and vegetables if they had begun to give the infant complementary foods or were interested to do so. In general mother-infant pairs in routine care at the well-baby clinics do not attend for an additional visit at 4 months of age and therefore the mothers in this cohort did not receive any advice on complementary feeding from a professional until 5 months of infant's age when they had already been eating complementary foods for a month.

All mothers who had unlimited access to lactation consultants and were randomized to give their infant complementary foods from 4 months of age in addition to breast milk, were thoroughly advised about the complementary feeding in an additional 4 month visit by a lactation consultant. The lactation consultants gave similar advice to that given in routine care at the well-baby clinics, i.e., to give the infant mainly porridges and purées of fruits and vegetables. Yet, the lactation consultants emphasized to the mothers the importance of giving diverse complementary foods and that breast milk should be the main food in the infant's diet until 6 months of age; mothers were therefore advised to give their infants small amounts of complementary foods and that one teaspoon once a day was enough until 6 months. The

mothers with unlimited access to lactation consultants received more nutritional support during the initial complementary feeding period than is usually provided in routine care at the well-baby clinics.

### **Data collection**

Dietary data was collected in both cohorts. Mothers of infants receiving complementary foods from 4 months of age and having unlimited access to lactation consultants obtained a 3 day weighed food records when the infant reached approximately 5 months and 1 week ( $\pm 7$  days) of age. Foods were weighed on accurate electronic scales with 1 g accuracy (Philips HR 2385, Philips Corporation, Hungary) provided to mothers by the research staff. Furthermore, parents recorded the date that every new food item and a new meal were added to the infant's diet at the time period 4-6 months. Information was retrospectively collected on total duration of breastfeeding for all infants.

Among mother-infant pairs receiving routine care at the well-baby clinics, dietary data was collected monthly from 5-8 months by 24-hour food records, where mothers recorded all foods the infant consumed. Furthermore, information on total duration of breastfeeding was collected retrospectively for all infants. Comparable dietary data for the two study groups were for ages 5 and 6 months.

### **Statistical analysis**

Statistical analyses were performed using the SPSS Windows statistical software package version 20.0 (SPSS Inc., Chigaco, IL, USA). Means and standard deviations were used to describe normally distributed variables while median and interquartile range was used for skewed variables. For comparison between two groups, independent t-test was used for normally distributed data and non-parametric Mann-Whitney U test when data were not



normally distributed. Chi-square tests of association and two-sided Fisher's exact test was used to compare dichotomous variables. A one-way ANOVA and Bonferroni Post Hoc Test were used to assess the differences in total breastfeeding duration. The level of significance in the study was set at  $P \leq 0.05$ .

## RESULTS

### Sample size and characteristics of subjects

A total of 50 mother-infant pairs who had unlimited access to lactation consultants and a total of 28 mother-infant pairs who received routine care at well-baby clinics were included in the primary data analysis for those infants who received complementary foods from 4 months of age in addition to breast milk and also in the secondary data analysis. A total of 50 mother-infant pairs who had unlimited access to lactation consultant and 15 mother-infant pairs who received routine care at well-baby clinics were included in the secondary data analysis for those who were exclusively breastfed for 6 months. No significant differences were seen in the characteristics between subjects of the study groups, as shown in **Table 1**.

### Dietary diversity and number of meals

**Table 2** compares the number of types of solid, semi-solid and soft foods infants consumed over 24 hours at approximately 5 months of age in the 2 study populations. Higher proportion of infants whose mothers had unlimited access to lactation consultants consumed some vegetables and vegetable purées ( $p = 0.05$ ) in these 24 hours, furthermore a greater proportion of them consumed more than 1 food type ( $p = 0.05$ ) compared with mother-infant pairs who received routine care at the well-baby clinics. Food types of solid, semi-solid and soft foods

that infants consumed at 5 months were solely porridges, fruits & fruit purées, vegetables & vegetable purées, oils and butter in both study populations. **Table 3** compares number of infants receiving specific number of meals daily at 5 and 6 months of age in the two study groups. At 5 months of age there was no significant difference seen in number of infants receiving specific number of meals daily between the two groups, however at 6 months a significantly higher number of infants receiving routine care at the well-baby clinics had three meals per day ( $p = 0.001$ ) compared with infants of mothers having unlimited access to a lactation consultant.

### **Total breastfeeding duration**

Comparison of total breastfeeding duration of infants receiving complementary foods from 4 months and those exclusively breastfed for 6 months in each of the two study populations were performed. Total breastfeeding duration was logarithmically transformed because of its skewed distribution and it differed significantly between the 4 study groups,  $F(3, 133) = 5.26$ ,  $p = 0.002$ . Bonferroni post-hoc comparisons indicated that infants exclusively breastfed for 6 months whose mothers had unlimited access to lactation consultants ( $1.08 \pm 0.15$ ;  $n=50$ ) had significantly longer total breastfeeding duration than infants who received complementary foods from 4 months of age, both among those who received routine care at the well-baby clinics ( $0.96 \pm 0.13$ ;  $n=26$ , 95% CI 0.03, 0.22) and those who had unlimited access to lactation consultants ( $1.01 \pm 0.11$ ;  $n=49$ , 95% CI -0.001, 0.16). They did however not have longer total breastfeeding duration than infants exclusively breastfed for 6 months and whose mothers received routine care at the well-baby clinics ( $1.08 \pm 0.27$ ;  $n=12$ , 95% CI -0.12, 0.14). No other significant differences were seen between groups.

**Figure 1** shows breastfeeding frequency at every age in the study populations. Half of the mothers who exclusively breastfed their infants for 6 months breastfed their infants for 12 months or beyond, both among those who received unlimited access to lactation consultants (50%) or routine care at the well-baby clinics (50%). 27% and 35% of mothers who introduced complementary foods from 4 months of age were still breastfeeding their infants at 12 months of age among those receiving routine care at the well-baby clinics and having unlimited access to lactation consultants, respectively. Only infants of mothers who were exclusively breastfed for 6 months were breastfed up to or beyond 2 years, both among those receiving unlimited access to lactation consultants (8%) and those receiving routine care at the well-baby clinics (14%).

## DISCUSSION

At approximately 5 months of age, a greater proportion of infants whose mothers had unlimited access to lactation consultants consumed some vegetables and vegetable purées over 24 hours and a higher proportion of them had more than one food type in their diet at this age. The mothers who had unlimited access to lactation consultants had slower introduction of complementary foods, according to number of infant's meals at 6 months, than mothers receiving routine care at the well-baby clinics.

The importance of adequate diet in infancy has long been recognized and it has become evident that early nutrition can affect the development of diseases later in life. <sup>[22-24]</sup> Infants receiving excessive complementary foods at a young age might be at risk of too rapid weight gain in infancy which could furthermore increase the risk of being overweight or obese in childhood and adulthood. <sup>[15]</sup> Because of the well substantiated advantages of breast

milk, recommendations for the introduction of complementary foods of breastfeeding children should take into account the effect of complementary feeding practices on breast milk intake.

<sup>[25]</sup> National recommendations for minimum dietary diversity and meal frequency do not exist for Icelandic or US infants younger than 6 months. Some studies suggest that fewer meals and therefore probably higher breast milk intake might be beneficial for infants at this age. <sup>[25,26]</sup> It should be noted that at 6 months, information on dietary intake of infants whose mothers received unlimited access to lactation consultants was derived from food diaries and it is thus possible that caregivers may have forgotten to record a meal in the food diary and therefore the frequency of meals could be underestimated. Furthermore, it is likely that many of the mothers with unlimited access to lactation consultants were interested in nutrition and breastfeeding, therefore the results of the current study might not be generalizable for the whole population.

Slower introduction of complementary foods, according to number of infant's meals at 6 months of age, among mothers having unlimited access to lactation consultants might be explained by the intervention itself since those mothers were randomized in either of the two groups and might therefore not see as much benefit of giving complementary foods, especially in big amounts, compared with mothers receiving routine care. Mothers receiving routine care at the well-baby clinics had generally chosen to begin complementary feeding before 6 months for a particular reason, e.g. their infant appeared to be hungry, had trouble sleeping and was waking up more often during the night (Unpublished data) and might therefore have had more reason for faster introduction of complementary foods. This might weaken the theory that the observed difference in introduction of complementary foods is primarily related to the exposure to lactation consultants. Yet, having easy access to lactation consultants may improve the quality of advice to mothers and give them the confidence to

continue to breastfeed, despite encountered problems. Further studies need to be done on the impact of advises by lactation consultants on the initiation of complementary feeding.

In the present study we found that infants whose mothers received unlimited access to lactation consultants and were exclusively breastfed for 6 months had longer duration of breastfeeding compared with the infants receiving complementary foods from 4 months of age in both study groups. Additionally, no difference was seen in total duration of breastfeeding between the two study groups whose mothers exclusively breastfed their infants for 6 months. The prevalence rate of exclusive breastfeeding for 6 months is low globally, both in resource rich and constrained countries. <sup>(27)</sup> It is possible that more exposure to lactation consultants the first months of infant's life would increase the prevalence of mothers exclusively breastfeeding for 6 months, and according to the results of the current study, may therefore have more prolonged breastfeeding. Maternal age and education are known to positively affect duration of breastfeeding, <sup>[28-32]</sup> but in the current study those factors were similar at baseline and therefore not considered to be confounding factors in the outcome.

The WHO recommends breastfeeding together with complementary foods until 2 years of age or beyond <sup>[33]</sup> and the American Academy of Pediatrics recommends breastfeeding for 1 year or beyond. <sup>[34]</sup> Breastfeeding support is important for mothers so they can achieve these recommendations and longer duration of exclusive and total breastfeeding. Results from the present study indicate that exclusive breastfeeding for 6 months appears to support longer total breastfeeding duration compared to exclusive breastfeeding for 4 months, regardless of exposure to lactation consultants. In the present study only mothers who exclusively breastfed their infants for 6 months achieved the WHO recommendations.

While the health benefits of breastfeeding are well known<sup>[34,35]</sup> less is known about the benefits of prolonged breastfeeding beyond one year of age. For example, more prolonged breastfeeding has been shown to be associated with a lower incidence of childhood infections.<sup>[36-38]</sup> It remains to be established whether a longer duration of breastfeeding is associated with a decreased risk of overweight and obesity in childhood and beyond.<sup>[39-44]</sup> Since exclusive breastfeeding for 6 months can increase the total breastfeeding duration compared with infants exclusively breastfed for 4 months, this may have significant benefits beyond the period of infancy.

## **CONCLUSION**

The results of this study suggest that mothers with unlimited access to lactation consultants have slower introduction of complementary foods at the initial complementary feeding period, according to number of infant's meals at 6 months of age. Furthermore, infants exclusively breastfed for 6 months seem to have longer total breastfeeding duration compared with those receiving complementary foods from the age of 4 months, irrespective if the families receive routine care at the well-baby clinics or have unlimited access to lactation consultants. Mothers with more exposure to lactation consultants might become more confident and likely to follow advices given about complementary feeding compared with those receiving routine care at the well-baby clinics. Further studies are needed to determine the generalizability of these findings and their long term effect on the dietary patterns and food choices of young children.

## **COMPETING INTERESTS**

The initial studies were funded by Mead Johnson, the Eimskip Fund of the University of Iceland and the Icelandic Research Fund for graduate students. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, preparation of the report or the decision to submit for publication. The study was also supported by the Primary Health Care Organisations in Reykjavík Capital Area, Akranes and Sudurnes and the directors of the participating health centres. All authors had full access to the trial data for interpretation. The corresponding author was responsible for the final decision to submit the report for publication. The authors have indicated they have no financial relationship relevant to this article to disclose. MF has received research funding from and undertaken advisory work for companies manufacturing infant formulas and baby foods within the past 3 years, all the other authors declare that they have no conflicts of interest.

## **AUTHORS CONTRIBUTIONS**

The present study was structured and designed by IT, MF, GG, RK and PH. OJ was responsible for the data collection in the randomized controlled trial and, following extensive discussions amongst all authors, conducted the statistical analysis and wrote the first draft of the manuscript. JJ, IE and AR participated in the planning of the data collection and its collection and contributed the manuscript regarding the lactation consultants and their contact with the mothers in the study. The manuscript was critically reviewed and revised by all authors.

## ACKNOWLEDGEMENT

We acknowledge all participants in the study. The lactation consultants Elin Sigurbjornsdottir, Gudrun Gudmundsdottir and the nurses Audur Egilsdottir, Dagny Gudmundsdottir, Kristin J. Vigfusdottir and Sesselja Gudmundsdottir are acknowledged for recruiting mother-infant pairs and their great amount of assistance in data collection. Also we thank the dietician Asa Vala Thorisdottir for her assistance in data analysis.

## REFERENCES

1. World Health Organization (WHO). **Feeding and nutrition in infants and young children. Guidelines for the WHO European Region, with emphasis on the former Soviet countries.** WHO Regional Publications, European Series, No. 87. Denmark, 2000.
2. Lucas A. **Programming by early nutrition in man.** *Ciba Found Symp* 1991, **156**, 38–50.
3. Mahrshahi S, Battistutta D, Magarey A, Daniels LA. **Determinants of rapid weight gain during infancy: baseline results from the NOURISH randomised controlled trial.** *BMC Pediatr* 2011, **7**(11):99.
4. Lamb MM, Dabelea D, Yin X, Ogden LG, Klingensmith GJ, Rewers M, Norris JM. **Early-life predictors of higher body mass index in healthy children.** *Ann Nutr Metab* 2010, **56**(1):16-22.
5. Griffiths LJ, Smeeth L, Hawkins SS, Cole TJ, Dezateux C. **Effects of infant feeding practice on weight gain from birth to 3 years.** *Arch Dis Child* 2009, **94**(8):577-82.
6. Gillman MW, Rifas-Shiman SL, Camargo CA Jr, Berkey CS, Frazier AL, Rockett HR, Field AE, Colditz GA. **Risk of overweight among adolescents who were breastfed as infants.** *JAMA* 2001, **285**(19):2461-7.
7. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. **Breast feeding and obesity: cross sectional study.** *BMJ* 1999, **319**(7203):147-50.
8. de Oliveira MI, Camacho LA, Tedstone AE. **Extending breastfeeding duration through primary care: a systematic review of prenatal and postnatal interventions.** *J Hum Lact* 2001, **17**(4):326-43.
9. Thurman SE, Allen PJ. **Integrating lactation consultants into primary health care services: are lactation consultants affecting breastfeeding success?** *Pediatr Nurs* 2008, **34**(5):419-25.
10. Castrucci BC, Hoover KL, Lim S, Maus KC. **A comparison of breastfeeding rates in an urban birth cohort among women delivering infants at hospitals that employ and do not employ lactation consultants.** *J Public Health Manag Pract* 2006, **12**(6):578-85.



11. Bonuck KA, Trombley M, Freeman K, McKee D. **Randomized, controlled trial of a prenatal and postnatal lactation consultant intervention on duration and intensity of breastfeeding up to 12 months.** *Pediatrics* 2005, **116**(6):1413-26.
12. Brent NB, Redd B, Dworetz A, D'Amico F, Greenberg JJ. **Breast-feeding in a low-income population. Program to increase incidence and duration.** *Arch Pediatr Adolesc Med* 1995, **149**(7):798-803.
13. Rosen IM, Krueger MV, Carney LM, Graham JA. **Prenatal breastfeeding education and breastfeeding outcomes.** *MCN Am J Matern Child Nurs* 2008, **33**(5):315-9.
14. Chung M, Ip S, Yu W, Raman G, Trikalinos T, DeVine D, Lau J. **Interventions in Primary Care to Promote Breastfeeding: A Systematic Review.** Prepared for the Agency for Healthcare Research and Quality by the Tufts-New England Medical Center Evidence-based Practice Center. Report No.09-05126-EF-1. Rockville (MD): Agency for Healthcare Research and Quality, 2008.
15. Lanigan J, Singhal A. **Early nutrition and long-term health: a practical approach.** *Proc Nutr Soc* 2009, **68**(4):422-9.
16. Wells JC, Jonsdottir OH, Hibberd PL, Fewtrell MS, Thorsdottir I, Eaton S, Lucas A, Gunnlaugsson G, Kleinman RE. **Randomised controlled trial of 4 versus 6 months exclusive breast-feeding in Iceland: differences in breast-milk intake by stable isotope probe.** *Am J Clin Nutr* 2012, **96**(1):73-9.
17. Jonsdottir OH, Thorsdottir I, Hibberd PL, Fewtrell MS, Wells JC, Palsson GI, Lucas A, Gunnlaugsson G, Kleinman RE. **Timing of the introduction of complementary foods in infancy: A randomized controlled trial.** *Pediatrics* published online: November 12, 2012 (doi:10.1542/peds.2011-3838).
18. Thorisdottir AV, Thorsdottir I, Palsson GI. **Nutrition and Iron Status of 1-Year Olds following a Revision in Infant Dietary Recommendations.** *Anemia*. 2011:986303. Epub 2011 Jul 18.
19. World Health Organization (WHO). **Indicators for assessing breastfeeding practices. Report of an informal meeting.** Division of Child and Adolescent Health. WHO/CDD/SER/91.14.ed. Geneva, World Health Organization, 1991. Available at [http://www.emro.who.int/cah/pdf/bf\\_indicators.pdf](http://www.emro.who.int/cah/pdf/bf_indicators.pdf).
20. Handbook for infant care in Iceland – Guidelines for healthcare of 0-5 year-olds. Directorate of Health, 2010. Available at <http://www.landlaeknir.is/servlet/file/store93/item2830/version6/4138.pdf>
21. The Icelandic nutrition council and centre for child health services, **Nutrition: the first twelve months**, The Icelandic Nutrition Council, Reykjavík, Iceland, 2003. Available at [http://www.landlaeknir.is/servlet/file/store93/item11416/version5/Baklingur\\_-\\_Naring\\_ungbarna\\_enska.pdf](http://www.landlaeknir.is/servlet/file/store93/item11416/version5/Baklingur_-_Naring_ungbarna_enska.pdf)
22. Berenson GS. **Childhood risk factors predict adult risk associated with subclinical cardiovascular disease. The Bogalusa Heart Study.** *Am J Cardiol* 2002, **90**(10C):3L-7L.
23. Caballero B. **Early nutrition and risk of disease in the adult.** *Public Health Nutr* 2001, **4**:1335-6.

24. Guo SS, Wu W, Chumlea WC, Roche AF. **Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence.** *Am J Clin Nutr* 2002, 76(3):653-8.
25. PAHO. **Guiding principles for complementary feeding of the breastfed child.** Washington, D.C.: Pan American Health Organization, World Health Organization, 2003. Available at <http://whqlibdoc.who.int/paho/2003/a85622.pdf>
26. Islam MM, Khatun M, Peerson JM, Ahmed T, Mollah MA, Dewey KG, Brown KH. **Effects of energy density and feeding frequency of complementary foods on total daily energy intakes and consumption of breast milk by healthy breastfed Bangladeshi children.** *Am J Clin Nutr* 2008, 88(1):84-94.
27. World Health Organization: *Infant and young child feeding: Model Chapter for textbooks for medical students and allied health professionals.* Geneva, WHO; 2009.
28. Dubois L, Girard M. **Social inequalities in infant feeding during the first year of life. The Longitudinal Study of Child Development in Québec (LSCDQ 1998-2002).** *Public Health Nutr* 2003, 6(8):773-83.
29. Michaelsen KF, Larsen PS, Thomsen BL, Samuelson G. **The Copenhagen cohort study on infant nutrition and growth: duration of breast feeding and influencing factors.** *Acta Paediatr* 1994, 83(6):565-71.
30. Hörnell A, Aarts C, Kylberg E, Hofvander Y, Gebre-Medhin M. **Breastfeeding patterns in exclusively breastfed infants: a longitudinal prospective study in Uppsala, Sweden.** *Acta Paediatr* 1999, 88(2):203-11.
31. Haughton J, Gregorio D, Pérez-Escamilla R. **Factors associated with breastfeeding duration among Connecticut Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) participants.** *J Hum Lact* 2010, 26(3):266-73.
32. Gudnadottir M, Gunnarsson BS, Thorsdottir I. **Effects of sociodemographic factors on adherence to breastfeeding and other important infant dietary recommendations.** *Acta Paediatr* 2006, 95(4):419-24.
33. World Health Organization (WHO). **Infant and young child nutrition. Global strategy on infant and young child feeding.** WHO 55<sup>th</sup> World Health Assembly, 16.april 2002. A55/15. Available at [http://apps.who.int/gb/archive/pdf\\_files/WHA55/ea5515.pdf](http://apps.who.int/gb/archive/pdf_files/WHA55/ea5515.pdf)
34. Johnston M, Landers S, Noble L, Szucs K, Viehmann L. **Breastfeeding and the use of human milk.** *Pediatrics* 2012, 129(3):827-41.
35. World Health Organization (WHO). **The optimal duration of exclusive breastfeeding: A systematic review.** 2001 WHO/NHD/01.08. Geneva, Switzerland.
36. Fisk CM, Crozier SR, Inskip HM et al. **Breastfeeding and reported morbidity during infancy: findings from the Southampton Women's Survey.** *Matern Child Nutr* 2011, 7(1):61-70.
37. Duijts L, Ramadhani MK and Moll HA. **Breastfeeding protects against infectious diseases during infancy in industrialized countries. A systematic review.** *Matern Child Nutr* 2009, 5(3):199-210.

38. Kramer MS, Chalmers B, Hodnett ED et al. **Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus.** *JAMA* 2001, **285**(4):413-20.
39. Gubbels JS, Thijs C, Stafleu A et. al. **Association of breast-feeding and feeding on demand with child weight status up to 4 years.** *Int J Pediatr Obes* 2011, **6**(2-2):515-22.
40. Harder T, Bergmann R, Kallischnigg G, Plagemann A. **Duration of breastfeeding and risk of overweight: a meta-analysis.** *Am J Epidemiol* 2005, **162**(5):397-403.
41. Kramer MS, Matush L, Vanilovich I et al. **Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity and blood pressure at age 6.5 y: evidence from a large randomized trial.** *Am J Clin Nutr* 2007, **86**(6):1717-21.
42. Durmus B, van Rossem L, Duijts L et al. **Breast-feeding and growth in children until the age of 3 years: the Generation R Study.** *Br J Nutr* 2011, **105**(11):1704-1711.
43. Kitsantas P and Gaffney KF. **Risk profiles for overweight/obesity among preschoolers.** *Early Hum Dev* 2010, **86**(9):563-8.
44. Jiang M, Foster EM. **Duration of Breastfeeding and Childhood Obesity: A Generalized Propensity Score Approach.** *Health Serv Res* 2012, [Epub ahead of print]

## LEGENDS FOR ILLUSTRATIONS

**Table 1. Baseline characteristics of participants in the study.**

Characteristics of subjects and breastfeeding behavior at 5 months: mother-infant pairs who had unlimited access to lactation consultants (received complementary foods from 4 months: n=50; exclusively breastfed for 6 months: n=50) and mother-infant pairs who received routine care at the well-baby clinics (received complementary foods from 4 months: n=28; exclusively breastfed for 6 months: n=15).

Variable	Complementary feeding from 4 months of age		Exclusive breastfeeding for 6 months	
	Lactation consultant	Routine care	Lactation consultant	Routine care
Birth weight (kg)	3706 (448)	3745 (376)	3707 (518)	3816 (301) <sup>a</sup>
Length at birth (cm)	51.4 (1.8)	51.8 (1.6)	51.6 (2.0)	52.2 (1.3) <sup>a</sup>
Weight at 6 months (kg)	7961 (1058)	7978 (816) <sup>a</sup>	8005 (1046)	8136 (1077) <sup>a</sup>
Length at 6 months (cm)	68.2 (2.3)	68.7 (2.1) <sup>a</sup>	68.5 (2.2)	68.9 (1.9) <sup>a</sup>
Maternal age	29.7 (4.4)	31.2 (4.9)	30.7 (5.1)	32.6 (4.9) <sup>b</sup>
Maternal education at university level <sup>c,d</sup>	30 (60%)	12 (50%) <sup>b</sup>	24 (48%)	6 (55%) <sup>b</sup>
Breastfeeding frequency (times/24 hours)	8.2 (2.0)	8.7 (2.9)	-	-
Total time breastfeeding (min/24 hours)	69.5 (31.5)	78.9 (40.8)	-	-

Data are presented as mean (SD) unless otherwise indicated

<sup>a</sup>Two missing values (n=13, n=26)

<sup>b</sup>Four missing values (n=11, n=24)

<sup>c</sup>Finished studies at university level

<sup>d</sup>Data are presented as number (%)

**Table 2. Number of types of solid foods infants consumed at 5 months of age.**

Selected food types infants received at approximately 5 months among those who received complementary foods from the age of 4 months in addition to breast milk having unlimited access to lactation consultant (n=50) or received routine care at the well-baby clinics (n=28).

Number of food types	Lactation consultant	Routine care	P-value
Total number of food types	1.5 (1.0) <sup>b</sup>	1.0 (1.0) <sup>b</sup>	0.06
>1 food type	25 (50%)	7 (27%)	0.05
>2 food types	9 (18%)	2 (8%)	0.31
>3 food types	5 (10%)	2 (8%)	1.00
Porridge	40 (80%)	24 (92%)	0.20
>1 type	3 (6%)	2 (8%)	1.00
Vegetables & vegetable purées	11 (22%)	1 (4%)	0.05
>1 type	3 (6%)	0 (0%)	0.55
Fruit & fruit purées	17 (34%)	7 (27%)	0.53
>1 type	4 (8%)	1 (4%)	0.66

Data are presented as number (%) unless otherwise indicated

<sup>a</sup>Two missing values (n=26)

<sup>b</sup>Data are presented as median (IQR)

**Table 3. Number of meals the infants consumed daily at 5 and 6 months of age.**

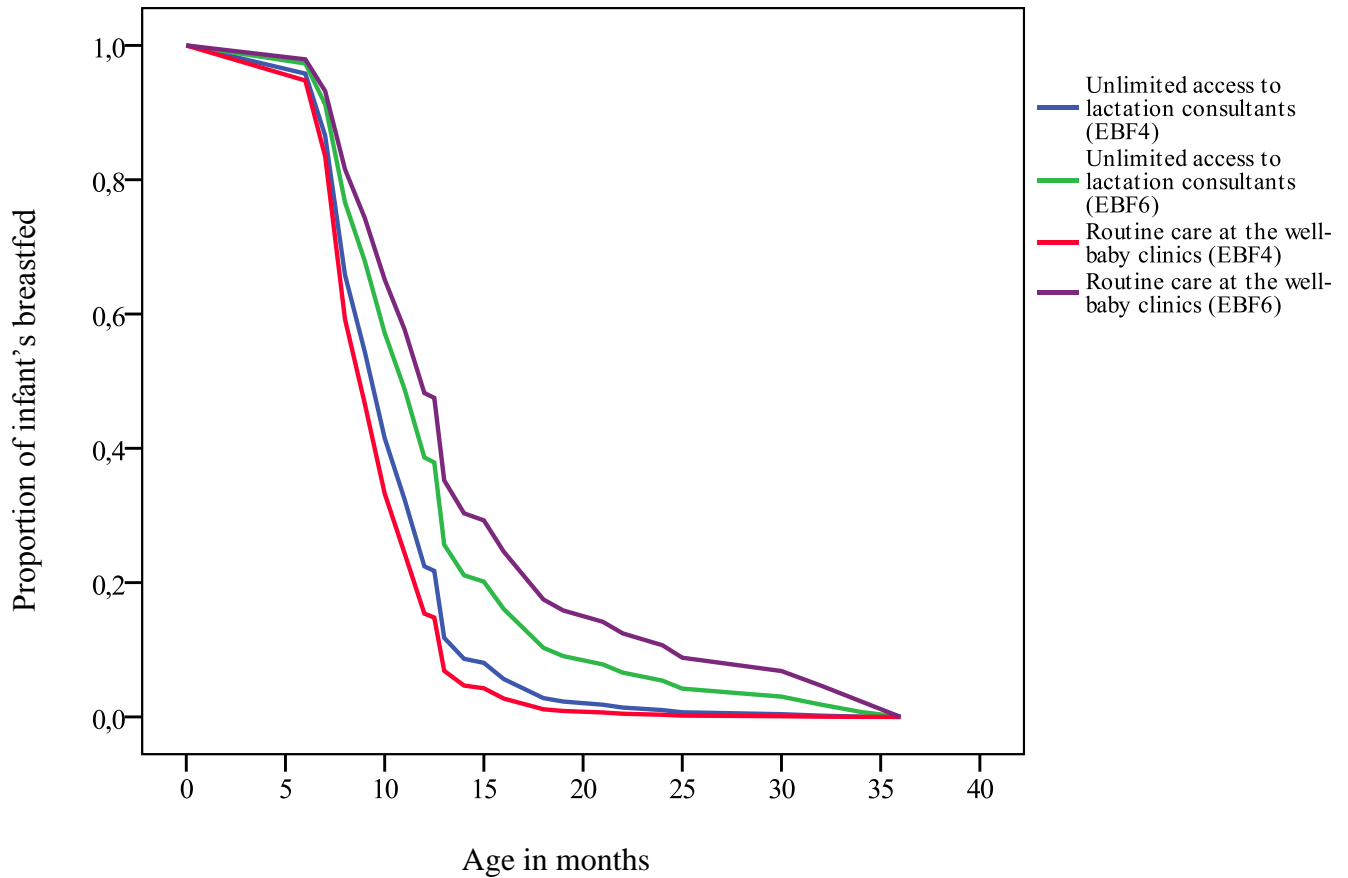
Number of meals at 5 and 6 months of age among infants receiving complementary foods from the age of 4 months in addition to breast milk, having unlimited access to lactation consultant (n=50) or received routine care at the well-baby clinics (n=28).

	5 months of age			6 months of age		
Daily meal frequency	Lactation consultant	Routine care	P-value	Lactation consultant	Routine care	P-value
One meal	34 (68%)	21 (81%)	0.24	18 (37%)	5 (19%)	0.10
Two meals	16 (32%)	3 (12%)	0.06	29 (59%)	11 (41%)	0.12
Three meals	0 (0%)	2 (8%)	0.11	1 (2%)	8 (30%)	0.001
Four meals	0 (0%)	0 (0%)	-	1 (2%)	3 (11%)	0.13

Data are presented as number (%)

<sup>a</sup>Two missing values (n=26)

<sup>b</sup>One missing value (n=49; n=27)



**Figure 1. Breastfeeding frequency at every age in the two study populations.**

Breastfeeding frequency at every age in the study populations i.e. infants whose mothers had unlimited access to lactation consultants (received complementary foods at 4 months, EBF4: n=49; exclusively breastfed for 6 months, EBF6: n=50) and those who received routine care at the well-baby clinics (received complementary foods at 4 months, EBF4: n=26; exclusively breastfed for 6 months, EBF6: n=12).