

**Supplemental Table 1: Older Sibling Information for Each of the 57 High-risk Infants in Our Study.**

Subject	Gender	Original Outside Clinical Diagnosis <sup>1,3</sup>	Age at Outside Clinical Diagnosis	Clinical Best Estimate Diagnosis <sup>2,3</sup>	Age at Clinical Best Estimate Diagnosis
S1	M	ASD	2 yrs, 4 mos	AD	6 yrs, 3 mos
S2	M	AD	2 yrs, 11 mos	PDD-NOS	5 yrs, 0 mos
S3	M	AD	2 yrs, 10 mos	AD	4 yrs, 4 mos
S4	M	AD/MR (unspecified)	2 yrs, 10 mos	AD	3 yrs, 11 mos
S5	M	AD	7 yrs, 5 mos	AD	7 yrs, 5 mos
S6	M	AD/MR (unspecified)	3 yrs, 4mos	AD	3 yrs, 10 mos
S7	M	AD	3 yrs, 4mos	PDD-NOS	7 yrs, 3 mos
S8	M	AD	6 yrs, 0 mos	AD	6 yrs, 11 mos
S9	M	GDD, ASD	1 yr, 9 mos	AD	3 yrs, 8 mos
S10	M	AD	2 yrs, 5 mos	PDD-NOS	3 yrs, 11 mos
S11	M	AD	3 yrs, 2 mos	AD	5 yrs, 1 mos
S12	M	AD	2 yrs, 11 mos	AD	3 yrs, 3 mos
S13	M	AD	4 yrs, 2 mos	AD (HF)	5 yrs, 10 mos
S14	F	AD	2 yrs, 11 mos	AD (HF)	5 yrs, 7 mos
S15	M	AD	2 yrs, 11 mos	AD (HF)	3 yrs, 5 mos
S16	M	ASD	2 yrs, 3 mos	AD	6 yrs, 4 mos
S17	M	PDD-NOS	3 yrs, 2 mos	PDD-NOS	4 yrs, 6 mos
S18	M	AD	4 yrs, 5 mos	AD	8 yrs, 4 mos
S19	M	PDD-NOS	2 yrs, 5 mos	PDD-NOS	3 yrs, 9 mos
S20	M	AD	2 yrs, 4 mos	AD	4 yrs, 5 mos
S21	M	AD	2 yrs, 11 mos	AD	4 yrs, 10 mos
S22	M	PDD-NOS	2 yrs, 4 mos	PDD-NOS	3 yrs, 2 mos
S23	M	AD	2 yrs, 6 mos	AD	3 yrs, 11 mos
S24	M	AD	2 yrs, 11 mos	AD	5 yrs, 3 mos
S25	M	ASP	4 yrs, 5 mos	PDD-NOS	5 yrs, 7 mos
S26	M	AD	2 yrs, 11 mos	AD	6 yrs, 9 mos
S27	M	ASD	2 yrs, 5 mos	PDD-NOS	2 yrs, 11 mos
S28	M	AD	2 yrs, 10 mos	AD	3 yrs, 10 mos
S29	M	AD	2 yrs, 11 mos	PDD-NOS	5 yrs, 7 mos
S30	M	PDD-NOS	3 yrs, 0 mos	AD	4 yrs, 1 mos
S31	F	AD	1 yr, 9 mos	AD	6 yrs, 1 mos
S32	M	AD	2 yrs, 6 mos	AD	8 yrs, 2 mos
S33	M	Possible PDD-NOS	3 yrs, 2 mos	ASD	5 yrs, 8 mos
S34	M	AD*	1 yr, 11mos	AD	3 yrs, mos
S35	M	AD	2 yrs, 9 mos	AD	3 yrs, 2 mos
S36	M	AD	2 yrs, 0 mos	AD	2 yrs, 8 mos
S37	M	PDD-NOS	1 yr, 7 mos	PDD-NOS*	2 yrs, 1 mos
S38	M	AD	1 yr, 7 mos	AD	2 yrs, 8 mos

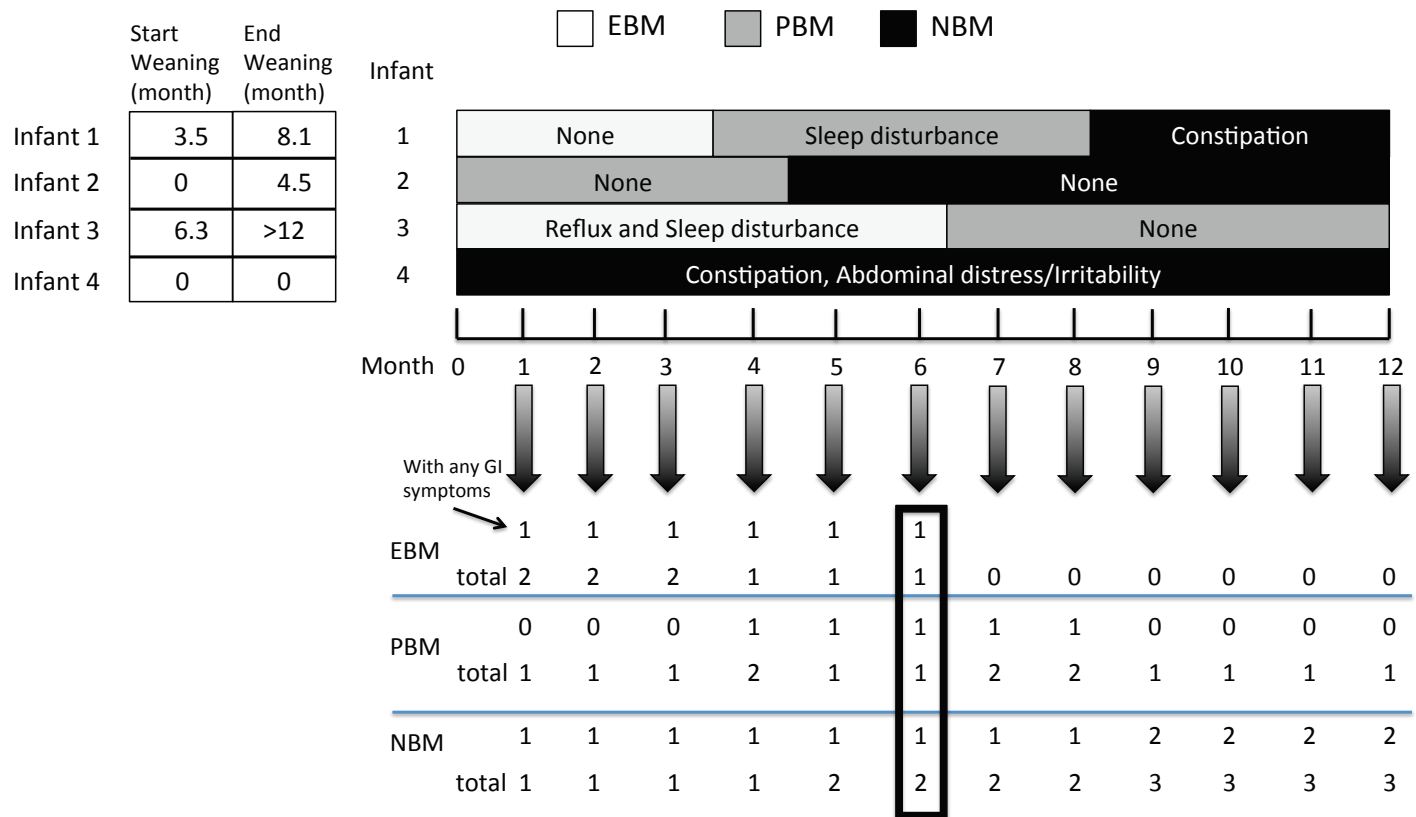
S39	M	Not Provided	Not Provided	AD	3 yrs, 11 mos
S40	M	AD	3 yrs, 3 mos	AD	3 yrs, 6 mos
S41	F	AD	2 yrs, 6 mos	AD	4 yrs, 10 mos
S42	M	ASD	1 yr, 6 mos	AD	3 yrs, 0 mos
S43	M	AD	2 yrs, 9 mos	AD	5 years, 8 mos
S44	M	AD	1 yr, 8 mos	AD	3 yrs, 9 mos
S45	M	AD	1 yr, 6 mos	AD*	2 yrs, 0 mos
S46	M	AD	3 yrs, 10 mos	AD	6 yrs, 4 mos
S47	F	AD	3 yrs, 6 mos	AD	13 yrs, 4mos
S48	M	AD	3 yrs, 11 mos	AD	7 yrs, 0 mos
S49	M	PDD-NOS	2 yrs, 0 mos	AD*	2 yrs, 2 mos
S50	M	AD	2 yrs, 8 mos	AD	7 yrs 6 mos
S51	M	AD	2 yrs, 10 mos	AD (HF)	3 yrs, 0 mos
S52	M	AD	2 yrs, 2 mos	AD	2 yrs, 4 mos
S53	M	AD	2 yrs, 6 mos	AD	5 yrs, 1mos
S54	M	ASD	4 yrs, 6 mos	AD	4 yrs, 11 mos
S55	M	ASD	4 yrs, 6 mos	AD	4 yrs, 11 mos
S56	M	PDD-NOS	3 yrs, 2 mos	PDD-NOS	4 yrs, 10 mos
S57	M	PDD-NOS	3 yrs, 0 mos	AD	5 yrs, 2 mos

<sup>1</sup> “Outside Clinical Diagnosis” was made by an outside clinical professional in the community, usually when the child was under age three.

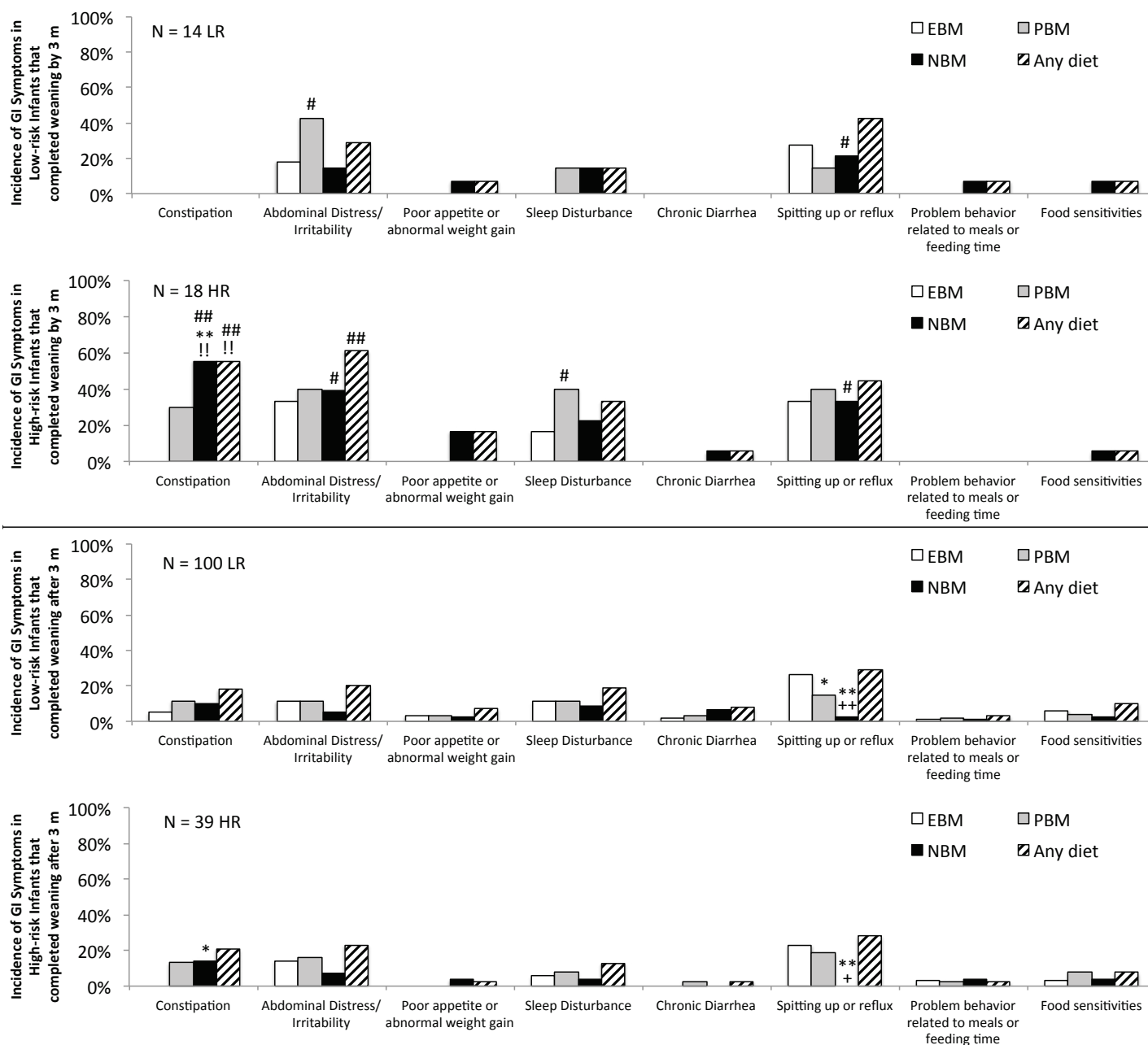
<sup>2</sup> A “Clinical Best Estimate Diagnosis” was made by our clinical psychologist (N. Akshoomoff) based on information from research diagnoses (ADOS and ADI-R) and clinical judgment using DSM-IV-TR criteria. There was sometimes a difference between the original outside clinical diagnosis and our Clinical Best Estimate Diagnosis, which is consistent with previous studies demonstrating that change in diagnosis is most likely to occur between the ages of two and five (Charman, et al., 2005; Lord, et al., 2006).

<sup>3</sup> AD = Autistic Disorder, ASP = Asperger's Disorder, PDD-NOS = Pervasive Developmental Disorder Not Otherwise Specified. HF = high functioning, MR = mental retardation (unspecified), GDD = Global Developmental Delay. \* = provisional diagnosis due to age.

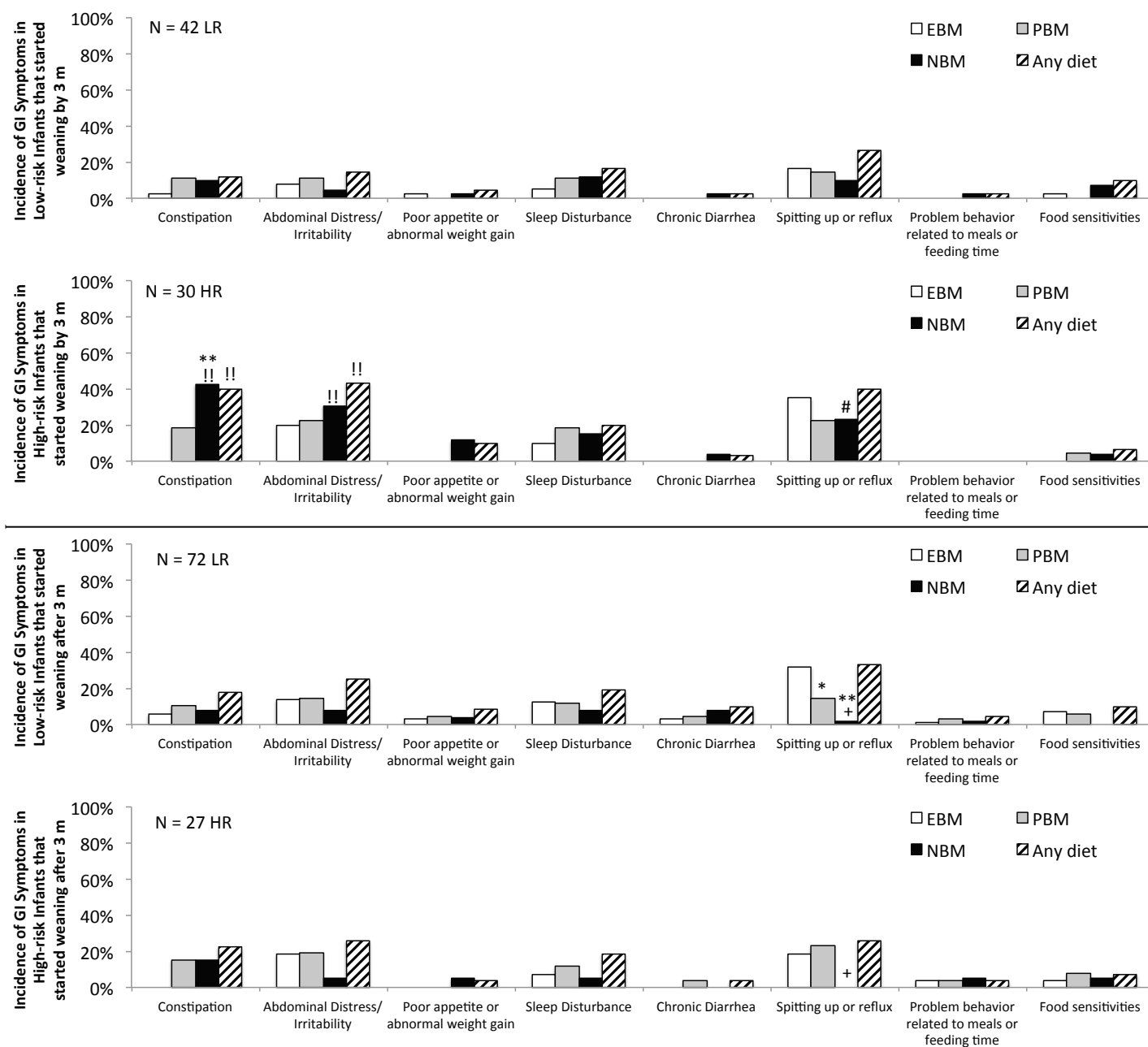
## Supplemental Figures



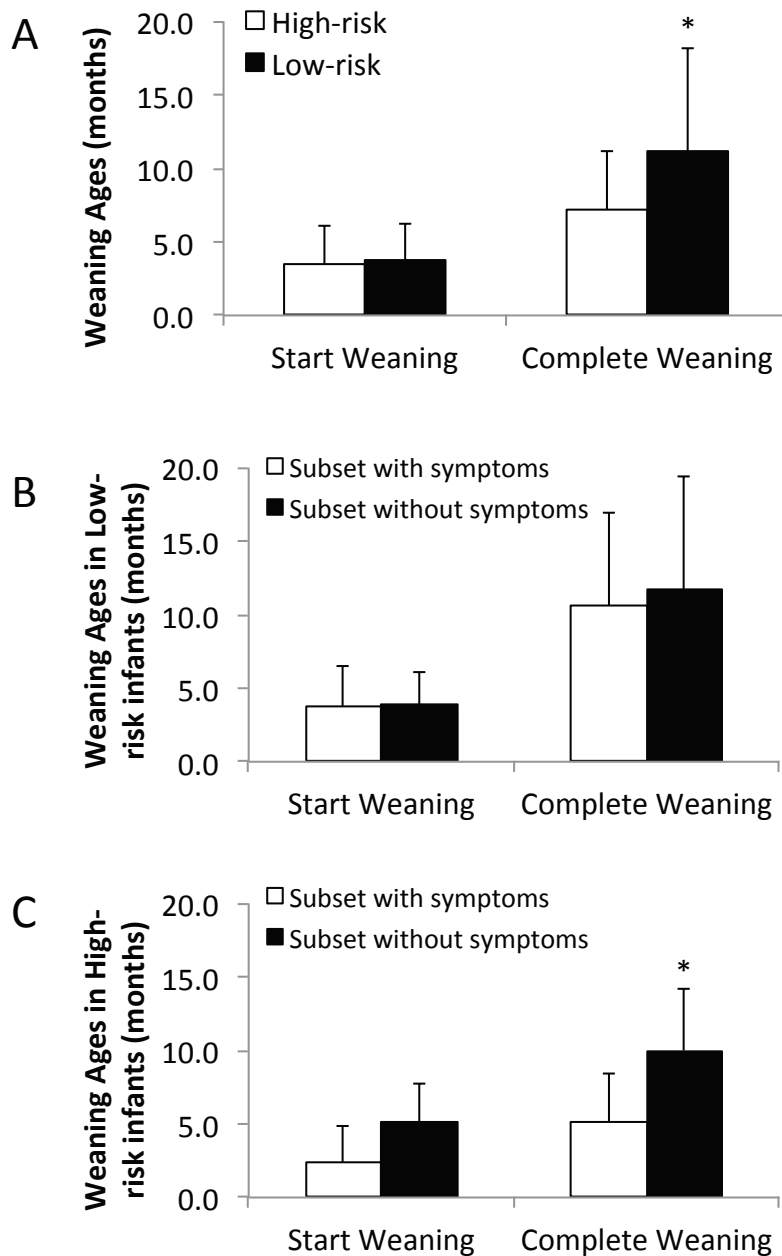
**Supplemental Figure 1.** Method for obtaining point prevalence of GI symptoms in infants during each diet category (numbers shown are hypothetical). Weaning ages are used to determine the number of infants in each diet category at each month of age. We count the infants in each diet category with any GI symptoms to determine prevalence. Infants with multiple symptoms are only counted once. For example, in this hypothetical data set, of the infants in the exclusive breast milk (EBM) category at 6 months, 100% (1 of 1) had a GI symptom reported for that diet category. Likewise, 100% (1 of 1) of partial breast milk (PBM) and 50% (1 of 2) of no breast milk (NBM) infants at that time had a GI symptom reported for their respective diet category.



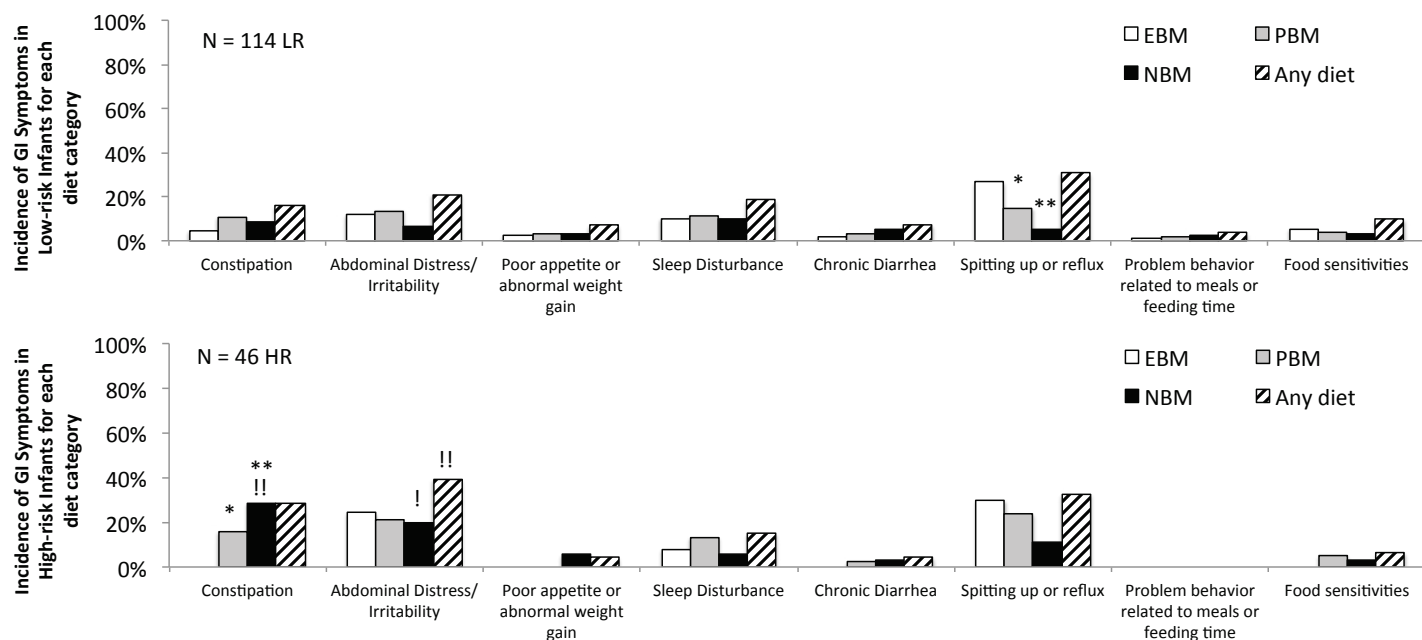
**Supplemental Figure 2.** Infants divided into those that completed weaning before versus after 3 months. \*\*  $p < 0.007$  NBM or PBM versus EBM, ++  $p = 0.007$  NBM versus PBM, !!  $p < 0.002$  High-risk versus Low-risk group, ##  $p < 0.013$  infants that completed weaning before versus after 3 months. As in Figure 4,  $\alpha = 0.013$  (Bonferroni-corrected Fisher test) for differences involving EBM, PBM, and NBM categories and  $\alpha = 0.025$  for the “Any diet” groups. Single symbols indicate marginal significance ( $\alpha < p < 0.05$ ).



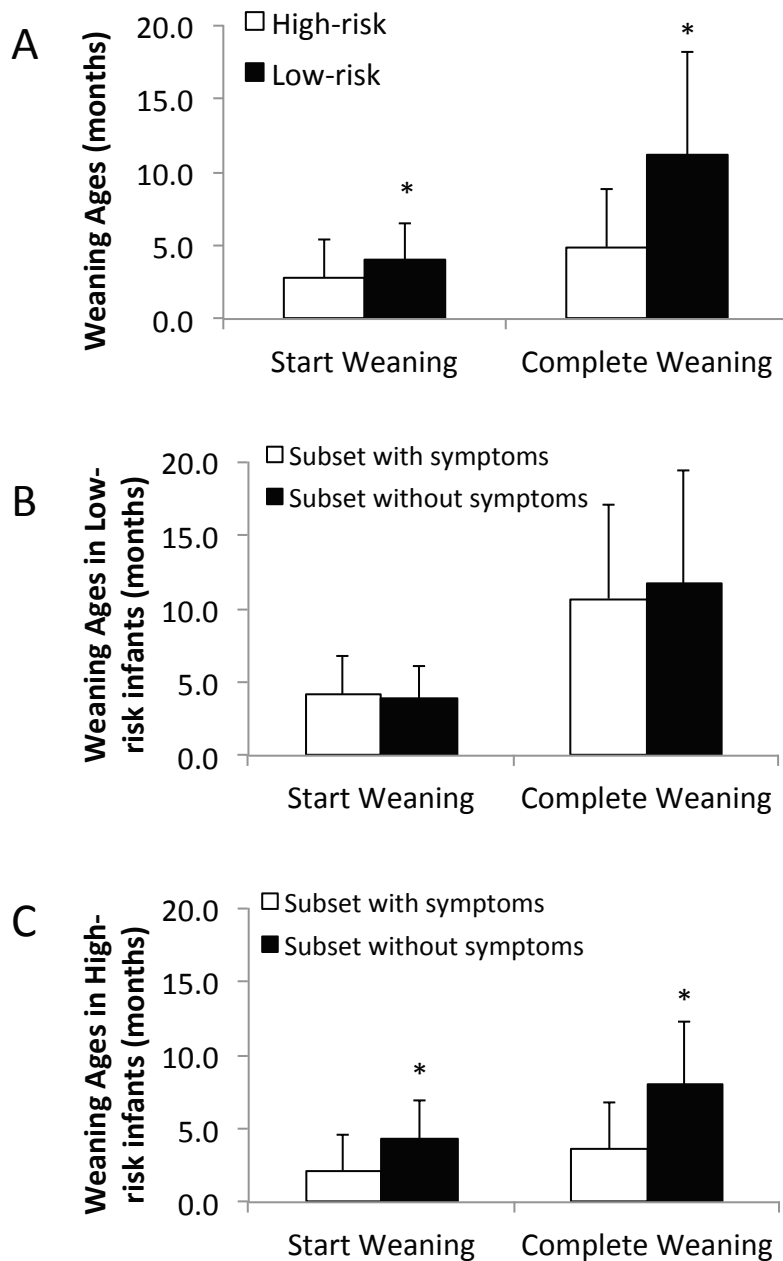
**Supplemental Figure 3.** Infants divided into those that started weaning before versus after 3 months. \*\*  $p < 0.001$  NBM or PBM versus EBM, ++  $p < \alpha$  NBM versus PBM, !!  $p < 0.008$  High-risk versus Low-risk group, ##  $p < \alpha$  infants that started weaning before versus after 3 months. As in Figure 4,  $\alpha = 0.013$  (Bonferroni-corrected Fisher test) for differences involving EBM, PBM, and NBM categories and  $\alpha = 0.025$  for the “Any diet” groups.



**Supplemental Figure 4** As in Figure 2, excluding infants diagnosed with ASD. (A) \*  $p < 0.004$  Low-risk versus High-Risk. (B&C) \*  $p < 0.04$  without versus with symptoms. N (start weaning) = 46 (28 with symptoms) High-risk and 114 (54 with symptoms) Low-risk infants. N (finish weaning) = 36 (21 with symptoms) High-risk and 100 (50 with symptoms) Low-risk infants. Note,  $\alpha = 0.05$  (t-tests, no Bonferroni correction).

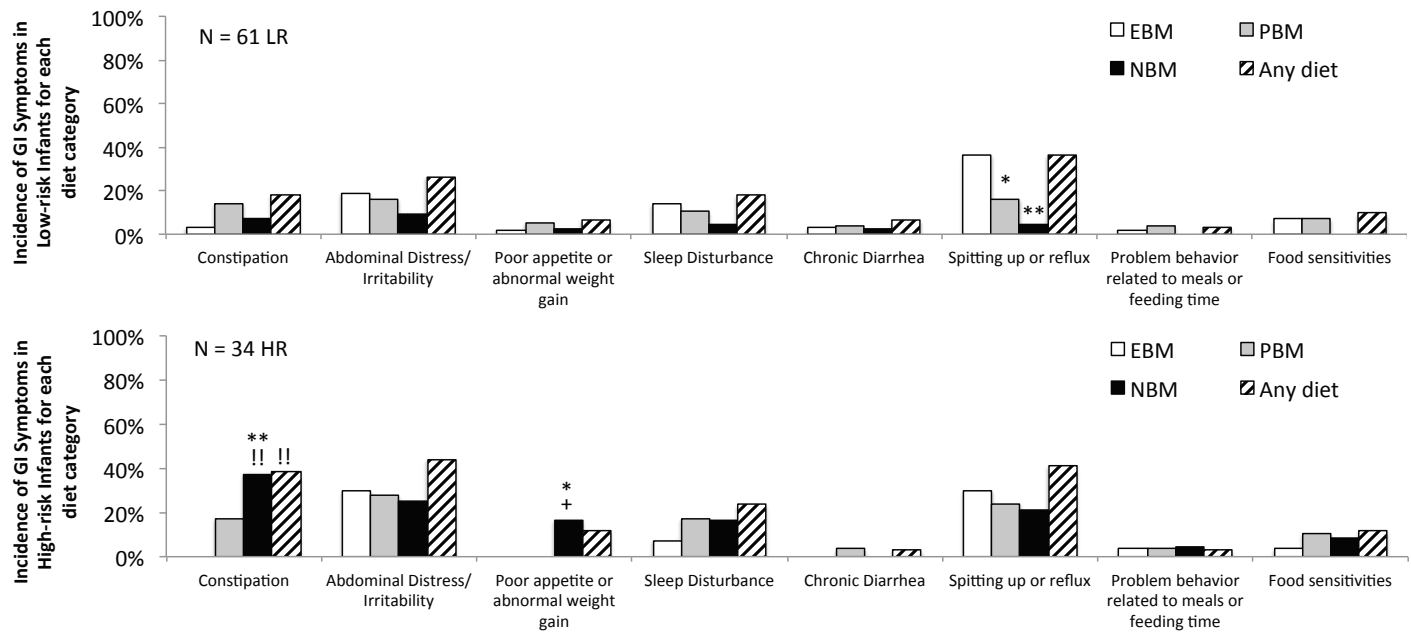


**Supplemental Figure 5** Symptom analysis (as in Figure 3) excluding infants diagnosed with ASD. \*\*  $p < 0.0004$  NBM or PBM versus EBM, !!  $p < \alpha$  High-risk versus Low-risk group. As in Figure 3,  $\alpha = 0.017$  (Bonferroni-corrected Fisher test) for differences involving EBM, PBM, and NBM categories and  $\alpha = 0.05$  for the “Any diet” groups. Single symbols indicate marginal significance ( $\alpha < p < 0.05$ ).



**Supplemental Figure 6** As in Figure 2, but excluding infants that were over 12 months of age at the time of their initial questionnaire to determine if recall bias is affecting findings. **(A)** \*  $p < 0.03$  Low-risk versus High-Risk. **(B&C)** \*  $p < 0.05$  without versus with symptoms. N (start weaning) = 34 (24 with symptoms) High-risk and 61 (34 with symptoms) Low-risk infants. N (finish weaning) = 24 (17 with symptoms) High-risk and 47 (24 with symptoms) Low-risk infants. Note,  $\alpha = 0.05$  (t-tests, no Bonferroni correction).





**Supplemental Figure 7** Symptom analysis (as in Figure 3) excluding infants that were over 12 months of age at the time of their initial questionnaire to determine if recall bias is affecting findings. \*\*  $p < 0.0005$  NBM or PBM versus EBM, ++  $p < \alpha$  NBM versus PBM, !!  $p < \alpha$  High-risk versus Low-risk group. As in Figure 3,  $\alpha = 0.017$  (Bonferroni-corrected Fisher test) for differences involving EBM, PBM, and NBM categories and  $\alpha = 0.05$  for the “Any diet” groups.

## Appendix A: Questionnaires

### GASTROINTESTINAL HEALTH AND DIET HISTORY (Initial Visit Questionnaire)

Today's date \_\_\_\_\_

Name of child \_\_\_\_\_

Date of birth of child \_\_\_\_\_

If at any point filling out this form you have questions, please ask the lab assistant!

First, has your child ever received breast-milk from a source other than the birth mother? Yes No

If yes, please explain \_\_\_\_\_

**How often does your child receive breast-milk? Circle the choice below that best applies:**

**Always** -- My child has never received anything but breast-milk or water (no formula/milk, juice, cereals or solid foods)

**Mostly** -- My child receives *predominately* breast-milk. But my child also receives formula/milk, juice, cereals, or solid foods (currently *less* than 5 servings per week)

**Sometimes** -- My child *sometimes* receives breast-milk. But my child also receives formula/milk, juice, cereals, or solid foods (currently 5 or *more* servings per week)

**Not currently** -- My child may or may not have received breast-milk in the past, but currently does not receive breast-milk.

If you are uncertain which category your child fits into, please explain below and fill out all **Sections** below that might apply to your child.

*Example: For medical reasons your child received a few formula feedings a few days after birth but afterwards has only received breast-milk (fill out Sections 1, 2, and 4).*

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If you answered **Always**, please fill out **Sections 1 and 4** below.

If you answered **Mostly** or **Sometimes**, please fill out **Sections 2 and 4** below.

If you answered **Not Currently**, please fill out **Sections 3 and 4** below.

#### **Section 1. Always Breast-Milk**

In a typical week, approximately what percentage of the time does your child breastfeed, that is, nurse directly from the breast (as opposed to receiving pumped/expressed/bottled breast-milk) \_\_\_\_\_ %?

If your child receives breast-milk that is pumped/expressed/bottled , in a typical week, about what % of the time is the pumped/expressed/bottled breast-milk given: (please add up to a total of 100%)

fresh (fed to baby immediately or stored for less than 1 day in a refrigerator) \_\_\_\_\_ %,

after refrigeration for more than 1 day \_\_\_\_\_ %

after freezing (for less than 1 week) \_\_\_\_\_ %

after freezing (for 1 week or more) \_\_\_\_\_ %?

In a typical week, do you supplement the breast-milk with water? Yes No

**Are you planning before your next visit (at \_\_\_\_\_ months) to introduce formula/milk, juice, cereals or solid foods as supplement to breast-milk, or switch completely to one of these other sources of nutrition?** Yes No

If yes, approximately when? \_\_\_\_\_

## **Section 2. Mostly or Sometimes Breast-Milk**

At what age did your child first receive breast-milk?

\_\_\_\_\_ days/weeks/months of age

At what age did your child first receive something other than breast-milk (formula/milk, juice, cereals or solid foods)?

\_\_\_\_\_ days/weeks/months of age

In a typical week, how many times is your child fed breast-milk? \_\_\_\_\_

In a typical week, how many times is your child fed something other than breast-milk? \_\_\_\_\_

Was there ever a period of time longer than 24 hours when your child did not receive breast-milk at all (that is, all servings were something other than breast-milk)? Yes No

If yes, please explain, and estimate number of days in a row

\_\_\_\_\_

In a typical week, how many servings of breast-milk are:

1) breastfed, that is, nursed directly from the breast? \_\_\_\_\_

2) pumped/expressed/bottled breast-milk? \_\_\_\_\_

If your child receives breast-milk that is pumped/expressed/bottled, in a typical week, about what % of the time is the pumped/expressed/bottled breast-milk given: (please add up to a total of 100%)

fresh (fed to baby immediately or stored for less than 1 day in a refrigerator) \_\_\_\_\_ %,

after refrigeration for more than 1 day \_\_\_\_\_ %,

after freezing (for less than 1 week) \_\_\_\_\_ %,

after freezing (for 1 week or more) \_\_\_\_\_ %?

What else does/did your child receive other than breast-milk? Circle all that apply:

juice, cow milk, soy milk, rice milk, cow milk-based formula, soy-based formula, other formula types,

pureed baby foods, table foods, other \_\_\_\_\_

If your child receives formula, what brand(s) do you currently use?

\_\_\_\_\_

What other brands have you tried in the past?

\_\_\_\_\_

**Are you planning before your next visit (at \_\_\_\_ months) to greatly decrease the frequency of breast-milk feedings or completely take your child off breast-milk? Yes No**

If yes, approximately when? \_\_\_\_\_

### **Section 3. Not Currently on Breast-Milk**

If your child never received breast-milk, check here ☐

If your child ever received breast-milk:

At what age did your child first receive breast-milk?

\_\_\_\_\_ days/weeks/months of age

At what age did your child first receive something other than breast-milk (formula/milk, juice, cereals or solid foods)?

\_\_\_\_\_ days/weeks/months of age

When did your child stop receiving breast-milk altogether? (answer may be same as above)

\_\_\_\_\_ days/weeks/months of age

What does your child currently receive? Circle all that apply:

juice, cow milk, soy milk, rice milk, cow milk-based formula, soy-based formula, other formula types, pureed baby foods, table foods, other \_\_\_\_\_

If you give formula, what brand(s) do you currently use?

\_\_\_\_\_  
What other brands have you tried in the past?

\_\_\_\_\_

#### Section 4. Sleep and Gastrointestinal Health History (please everyone fill out)

Has your child experienced any of the following symptoms to the degree that they were serious enough to seek medical advice or to prompt a change in your child's care? If so, please put an X in the box below that represents the type of diet your child was getting at the onset of the symptoms (see definitions of the four diet categories, *above*). If the symptoms persisted over different diet categories, mark all appropriate boxes (*for example, if your child experienced "sleep disturbance" while given "Always Breast-Milk", and also when given "Mostly Breast-Milk", put an X in both boxes.*)

If child never experienced any of these symptoms check here ☐

Symptoms	Always Breast-milk	Mostly Breast-milk	Sometimes Breast-milk	No Breast-milk
Sleep disturbance (circle the following that apply: cry/cough/restless)				
Chronic diarrhea (>3 loose stools/day for >2 weeks)				
Constipation (straining to pass stool, or hard/infrequent stools)				
Apparent abdominal discomfort/pain				
Gassiness and/or bloating				
Spitting up or reflux				
Unable to nurse or drink from a bottle				
Problem behavior related to meals or feeding time (please explain below)				
Poor appetite or abnormal weight gain				
Food sensitivities (please explain below)				
Colic (please explain below)				

Problem behavior related to meals or feeding time \_\_\_\_\_  
\_\_\_\_\_

Food sensitivities \_\_\_\_\_  
\_\_\_\_\_

Colic (please list all symptoms your child experienced: arching and screaming, constipation, throwing up, etc.) \_\_\_\_\_  
\_\_\_\_\_

## GASTROINTESTINAL HEALTH AND DIET HISTORY (Recurring Visit Questionnaire)

Today's date \_\_\_\_\_

Name of child \_\_\_\_\_

Date of birth of child \_\_\_\_\_

If at any point filling out this form you have questions, please ask the lab assistant!

First, since your last visit, has your child received breast-milk from a source other than the birth mother? Yes

No     If yes, please explain \_\_\_\_\_

**Since your last visit, how often has your child received breast-milk? Circle the choice below that best applies:**

**Always**        -- My infant/child has received nothing but breast milk or water since our last visit (no formula/milk, juice, cereals or solid foods)

**Mostly**        -- My child received *predominately* breast-milk in the time since our last visit. But my child also received formula/milk, juice, cereals, or solid foods (*less* than 5 servings per week)

**Sometimes**    -- My child has received breast milk **at least once** since our last visit. But my child also received formula/milk, juice, cereals, or solid foods (5 or *more* servings per week)

**None since last visit**    -- My child may or may not have received breast-milk in the past, but has not received breast-milk since our last visit.

If you answered **Always**, please fill out **Sections 1 and 4** below.

If you answered **Mostly** or **Sometimes**, please fill out **Sections 2 and 4** below.

If you answered **None since last visit**, please fill out **Sections 3 and 4** below.

### **Section 1. Always Breast-Milk**

In the time since your last visit, approximately what percentage of the time did your child breastfeed, that is, nurse directly from the breast (as opposed to receiving pumped/expressed/bottled breast-milk) \_\_\_\_\_%?

If your child received breast-milk that was pumped/expressed/bottled since your last visit, about what % of the time was the pumped/expressed/bottled breast-milk given: (please add up to a total of 100%)

fresh (fed to baby immediately or stored for less than 1 day in a refrigerator) \_\_\_\_\_%,

after refrigeration for more than 1 day \_\_\_\_\_%

after freezing (for less than 1 week) \_\_\_\_\_%

after freezing (for 1 week or more) \_\_\_\_\_%?

Since your last visit, did you supplement the breast-milk with water? Yes   No

Are you planning before your next visit (at \_\_\_\_\_ months) to introduce formula/milk, juice, cereals or solid foods as supplement to breast-milk, or switch completely to one of these other sources of nutrition? Yes No

If yes, approximately when? \_\_\_\_\_

## Section 2. Mostly or Sometimes Breast-Milk

How long ago did your infant **first** receive food other than breast milk or water? (only answer if occurred sometime after your last visit) \_\_\_\_\_ days/weeks/months ago

(If your infant received food other than breast milk for a brief period, went back to only breast milk for a while, and then began weaning again later, please answer the above question for the most recent start of weaning and explain here)

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Have you taken your child completely off of breast-milk in the time since your last visit? Yes No

If yes, when did that change occur? \_\_\_\_\_ days/weeks/months ago

In the time since your last visit:

In a typical week, how many times was your child fed breast-milk? \_\_\_\_\_

In a typical week, how many times was your child fed something other than breast-milk? \_\_\_\_\_

(If child taken completely off of breast-milk between this visit and the previous visit, answer for the period still on breast-milk)

Since your last visit, was there ever a period of time longer than 24 hours when your child did not receive breast-milk at all (that is, all servings were something other than breast-milk)? Yes No

If yes, please explain, and estimate number of days in a row

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In a typical week since your last visit, how many servings of breast-milk were:

1) breastfed, that is, nursed directly from the breast? \_\_\_\_\_

2) pumped/expressed/bottled breast-milk? \_\_\_\_\_

If your child received breast-milk that was pumped/expressed/bottled in the time since your last visit, about what % of the time was the pumped/expressed/bottled breast-milk given: (please add up to a total of 100%)

fresh (fed to baby immediately or stored for less than 1 day in a refrigerator) \_\_\_\_\_ %,

after refrigeration for more than 1 day \_\_\_\_\_ %

after freezing (for less than 1 week) \_\_\_\_\_ %

after freezing (for 1 week or more) \_\_\_\_\_ %?

What else did your child receive other than breast-milk? Circle all that apply:

juice, cow milk, soy milk, rice milk, cow milk-based formula, soy-based formula, other formula types, pureed baby foods, table foods, other \_\_\_\_\_

If your child receives formula, what brand(s) do you currently use?

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What other brands, if any, have you tried since your last visit?

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**Are you planning before your next visit (at \_\_\_\_ months) to greatly decrease the frequency of breast-milk feedings or completely take your child off breast-milk?** Yes No

If yes, approximately when? \_\_\_\_\_

### Section 3. None Since Last Visit

If you have taken your child completely off of breast-milk *in the time between your last visit and this visit*, please fill out *Section 2* instead. If your child did not receive any breast milk since their last visit, continue.

What does your child currently receive? Circle all that apply:

juice, cow milk, oy milk, rice milk, cow milk-based formula, soy-based formula, other formula types, pureed baby foods, table foods, other \_\_\_\_\_

If you give formula, what brand(s) do you currently use? \_\_\_\_\_

What other brands have you tried in the past? \_\_\_\_\_

### Section 4. Sleep and Gastrointestinal Health History (please everyone fill out)

Has your child experienced any of the following symptoms to the degree that they were serious enough to seek medical advice or to prompt a change in your child's care? If so, please put an X in the box below that represents the type of diet your child was getting at the onset of the symptoms (see definitions of the four diet categories, *above*). If the symptoms persisted over different diet categories, mark all appropriate boxes (*for example, if your child experienced "sleep disturbance" while given "Always Breast-Milk", and also when given "Mostly Breast-Milk", put an X in both boxes.*)

If child never experienced any of these symptoms check here ☐

Symptoms	Always Breast-milk	Mostly Breast-milk	Sometimes Breast-milk	No Breast-milk
Sleep disturbance (circle the following that apply: cry/cough/restless)				
Chronic diarrhea (>3 loose stools/day for >2 weeks)				
Constipation (straining to pass stool, or hard/infrequent stools)				
Apparent abdominal discomfort/pain				
Gassiness and/or bloating				
Spitting up or reflux				
Unable to nurse or drink from a bottle				
Problem behavior related to meals or feeding time (please explain below)				



<b>Poor appetite or abnormal weight gain</b>				
<b>Food sensitivities (please explain below)</b>				
<b>Colic (please explain below)</b>				

Problem behavior related to meals or feeding time \_\_\_\_\_  
 \_\_\_\_\_

Food sensitivities \_\_\_\_\_

Colic (please list all symptoms your child experienced: arching and screaming, constipation, throwing up, etc.) \_\_\_\_\_