Several problem nutrients are identified in the complementary diet of 6 to 11 month old breastfed children in Western Guatemala

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Running title: Problem nutrients in diets of Guatemalan infants

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ABSTRACT

Background and Objectives: The failure of infants in developing countries to meet nutrient intake recommendations is well documented. The objective of this study was to assess the nutritional adequacy and identify problem nutrients of the diets of Guatemalan infants with continued breastfeeding. **Methods and Study Design:** A single previous-day dietary recall was collected from a convenience sample of mothers of 94 infants aged 6-11 mo attending a public health clinic in the urban area of Quetzaltenango, Guatemala. Energy and nutrient content of complementary feeding (CF) and breastmilk, modelled by subtracting estimated energy intakes from CF from energy requirements, were calculated and nutrient adequacy of the diet was assessed. Nutrient densities and critical nutrient densities of CF were computed to identify "*problem nutrients*" and main food sources of these nutrients were identified. **Results:** Complementary diets were adequate for protein, but inadequate for pantothenic acid and vitamins C, A, D, E, and K, as well as calcium, iron and zinc. In the worst-case scenario, i.e. for small girls with limited energy allowances, riboflavin, niacin, vitamin B6 and magnesium were identified as "*problem nutrients*" as well. Formula milk, cow milk and *Incaparina®* were main food sources of "*problem nutrients*". **Conclusions:** The intake of micronutrients during the first six months of the recommend CF period in Guatemala has a number of notable inadequacies, but the gaps are narrower than traditionally reported for this age group in low-income settings.

Key Words: breastmilk, complementary feeding, infant feeding, micronutrient intake, Guatemala

INTRODUCTION

The WHO recommended transition from exclusive breastfeeding (for the first 6 mo of life) to family foods (at about 12 mo of age) is challenging, and high nutritional needs at this time period render infants particularly vulnerable to nutritional imbalances.[1] Infants require the consumption of nutrient-dense foods to cover the high nutritional requirements for life and growth relative to a small body size that thus, has limited energy requirements. The daily energy requirement from complementary feeding (CF), after allowing for breastmilk, is limited to 200 kcal at 6-8 mo and to 300 kcal at 9-11 mo.[2] The WHO has a series of "guiding principles" for CF that include specific recommendations to ensure nutrient adequacy. This includes age-specific recommendations for daily energy intake, number of meals, dietary variety and the use of vitamin A-rich fruits and fortified foods.[2]

Abundant survey literature from developing countries[3-8] and refined theoretical analysis and modeling[9-11] document the widespread failure to satisfy nutrient-intake recommendations for infant feeding; specifically for a family of "problem nutrients". These are defined as those for which there is most discrepancy between their content in CF and the amount required by the infant,[9] making them difficult to obtain without specific measures.

Within the comprehensive Xela-Babies study,[12-16] which examined feeding practices among infants and toddlers from contiguous urban zones in Quetzaltenango, we enrolled a cross-sectional sample of 94 infants aged between 6-11 mo. Daily intakes of CF were estimated using previous-day dietary recalls and assumed breastmilk consumption was modelled to estimate the overall diet. We hypothesized that we would find multiple nutrient gaps in the complementary diets of Guatemalan infants. We present here the description of average daily energy and micronutrient intakes consumed by the infants and nutrient densities in relation to international nutrient requirements, as well as leading food sources of the "*problem nutrients*".

MATERIALS AND METHODS

Subject selection

The study was conducted in the city of Quetzaltenango and the nearby suburb of La Esperanza, both located in the western highlands of Guatemala, in mothers of infants in the second semester of life. Initially 134 mother-child dyads were invited to participate while visiting local public health centres for routine check-ups, vaccinations or illness. A total of 20 mother-child dyads were excluded from the study for the following reasons: 1) pre-mature birth (defined as born more than 4 wk pre-term); 2) had siblings who were already participants; 3) had congenital anomalies or chronic illness; and 4) were unwilling to sign the study consent form. Finally, 114 (85% of those approached) mother-infant dyads were interviewed. The study was conducted between February and October, 2011.

Ethical approval was obtained from the Human Subjects Committee of the Center for Studies of Sensory Impairment, Aging and Metabolism in Guatemala (October 2010) and the Medical Ethics Review Committee of the VU University Medical Center in the Netherlands (IRB00002991, ref nb 2010/264). The study conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000). The protocol was registered at the Dutch trial registry "Nederlands Trialregister" (TC = 3292). The procedures and privacy issues of the study were explained to families of potential candidates and written informed consent was obtained from all mothers.

A structured questionnaire was administered by local, trained researchers to mothers by means of a face-to-face interview in Spanish. Information collected for the infant included: gender, date of birth, and place of birth. Data collection of socio-demographic characteristics of the mother included: date of birth, marital status, parity, highest level of education, and current occupation. Due to cultural sensitivities, direct questioning about ethnicity was not conducted; rather it was determined by observation of clothing used. Mayan Indigenous women usually wear traditional native clothing (huipiles and cortes).

Dietary intake was assessed by means of a single, previous-day dietary recall. A complete record of all foods and drinks consumed was collected. Food preparation methods including ingredients and brand names were asked and portion sizes were estimated using household measures. No previous-day recall was collected for infants who reportedly never consumed anything other than breastmilk, and these participants were excluded from the study. Breastmilk intake was not quantified by daily frequency.

Infant weight was measured to the nearest 100 g using a Tanita electronic scale (model BD-585, Tokyo, Japan). When the mother was unwilling to fully undress the child, the weight of each specific cloth item was deducted from the measured weight. Standard average weights of diapers and clothing were determined previously.

Data analysis

Energy and nutrient analysis of complementary feeding

All recipe and individual items portion sizes were converted from household measures into individual intake in grams. Standard portion sizes were previously determined. Their energy and nutrient values were primarily derived from USDA National Nutrient Database for Standard Reference version 26.[17] Nutrient values of food items not listed in the USDA table, such as lime-treated corn tortillas and refried black beans, were taken from a food composition compiled specifically for Latin foods.[18] Nutrition information not listed in the food databases or known to be different in Guatemala, such as vitamin A fortified table sugar, was taken from product labels or from information supplied by the manufacturer. Estimated daily intake of energy, water, protein, 12 vitamins (folate, pantothenic acid, thiamine, riboflavin, niacin, vitamin B-6, B-12, C, A, D, E, K) and 5 minerals (calcium, iron, magnesium, selenium, zinc) were computed.

Modelled intakes of breastmilk

Quantitative consumption of breastmilk was not evaluated. A simple approach to model the volume of breastmilk intake was developed and has been used previously by our team.[19-21] It is based on the assumption that the energy from breastmilk intake is equal to the energy requirement of the infant minus the energy derived from CF. Energy requirements were computed using the formula: -95.4 + 88.3 * body weight (kg), which takes into account daily energy requirement for growth.[22] The energy content of breastmilk was assumed to be 67 kcal/100 mL.[9] When the estimated energy from CF was higher than the energy requirement, the volume of breastmilk was arbitrarily set at zero.

Energy and nutrient analysis of breastmilk and total diet

The daily intake of energy and nutrient was computed as the sum of nutrients in CF plus the nutrients in modelled volumes of breastmilk. The micronutrient values of breastmilk were taken from average values reported for women in developing countries by Brown et al.[9] The water content of breastmilk was calculated based on the reported value of 87.5 g per 100 ml in the USDA National Nutrient Database for Standard Reference version 26.[17]

Nutrient adequacy

Estimated daily intake of protein was compared to the safe level of protein intake for infants of 1.3 g/kg.[23] Estimated daily intakes of 17 selected micronutrients were compared to Reference Nutrient Intakes (RNIs)[24] and expressed as percentages of requirements met. Since dietary estimates were based on a single previous-day recall, estimated distributions of usual intake of nutrients were adjusted for day-to-day variance using "*Intake Modelling, Assessment and Planning Program*" (IMAPP).[25] This software uses the variance in the diet using data from the National Health and Nutrition Examination Survey (NHANES)[26] and assumes similar variance in other populations. The proportion of infants meeting the RNIs for each nutrient was determined taking into account only CF or the total diet (i.e. CF plus modelled breastmilk).

Nutrient density of complementary feeding

The observed nutrient density of protein and 17 selected micronutrients in CF was calculated as the estimated nutrient intakes divided by the energy provided presented per 100 kcal for each age group.

Critical nutrient density

The critical nutrient density concept can be used to evaluate the nutrient adequacy of the diet. The critical nutrient density of CF is the nutrient level that would complement the assumed breastmilk consumption and can be used as a reference of adequate intakes. We used two different models, based on diverse assumption of breastmilk contribution, in our calculations.

Critical nutrient density based on distributional variable breastmilk-intake model

Theoretical CF nutrient gaps were calculated as the difference between the daily RNI values and the nutrient content of individually modelled volumes of breastmilk in our sample of 94 infants. Individual critical nutrient densities were computed as the theoretical CF nutrient gaps divided by the energy content of CF assessed by means of a single previous-day recall in our sample, and presented per 100 kcal.

Critical nutrient density based on fixed breastmilk energy contribution model

Critical nutrient density values for CF were calculated assuming a fixed breastmilk energy contribution as previously published by our group.[27,28] These were modelled at the group level to represent two extremes. Critical nutrient density values for boys with a median weight, i.e. following the 50th percentile of the 2006 WHO standards,[29] represented the best-case scenario, whereas values for girls with a low, but healthy, weight (15th percentile of the 2006 WHO standards), represented the worst-case scenario. Energy contribution from CF was assumed to be 25% in the 6 to 8 mo old infants and 50% in the 9-11mo old infants as recommended by the World Alliance for Breastfeeding Action (WABA).[30]

The median estimated nutrient densities of the observed diets were compared to the median critical densities based on variable breastmilk energy contribution and the critical nutrient densities published previously to identify "problem nutrients".[27,28]

Main dietary sources of "problem nutrients"

The ten leading sources of individual nutrients among the nine vitamins and minerals identified as "*problem nutrients*" in CF are presented in descending order of percent contribution to total daily intake.

Statistical analysis

Data were analysed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are presented. For internal and external (comparative publications) consistency, the arithmetic mean and standard deviation are presented, regardless of the normality of the distribution. The median is consistently presented and relied upon as the indication of central-tendency of record. No comparative statistical analysis was performed.

RESULTS

Response rate

Of the 134 mother-infant dyads initially approached to participate in the study, 114 (85%) mother-infant dyads were interviewed. A further 20 were excluded from the final data analysis; 12 did not breastfeed the day before the interview; and 8 breastfed exclusively on that day. The final sample size was 94 (70%).

Demographic characteristics

The demographic characteristics of the study population (n=94) are presented in Table 1. Although we aimed for equal distributions, there were fewer boys than girls, more participants from urban Quetzaltenango and more younger infants (6-8 mo) than older infants (9-11 mo) in the study.

Energy from complementary feeding and modelled volumes of breastmilk

As illustrated in Table 2, the estimated energy from CF jumped from a median of 237 kcal at 6-8 mo, covering 44% of the daily requirement, to a median of 351 kcal at 9-11 mo, contributing 58% of daily energy. For 13 infants (14%), the estimated daily energy intake from CF was higher than their energy requirement and the excess ranged from 52 to 385 kcal. The volume of breastmilk consumed by these children was artificially set at zero. The median modelled energy contribution from breastmilk was 331 kcal (range 0 to 620 kcal) that corresponds to 493 ml (range 0 to 926 ml) at 6-8 mo, and 224 kcal (range 0 to 748 kcal) and 335 ml (range 0 to 1117 ml) at 9-11 mo.

Estimated daily nutrient intakes and nutrient adequacy

The estimated daily nutrient intakes from CF based on a single previous-day recall, modelled volumes of breastmilk and total diet are presented in Table 3. As indicated on the last column of the table, the median estimated daily intake of pantothenic acid, vitamins C, D, E and K, calcium, iron and zinc (8 of 17 micronutrients) were below the RNIs. Median total water intake was 128 mL lower than the Adequate Intake (AI) for daily ingestion of the US-Canada Dietary Reference Intakes.[31]

As expected and illustrated in Table 4, with consumption disaggregated by age-group, estimated daily intakes of nutrients were generally higher in the older infants aged 9-11 mo than the younger infants aged 6-8 mo, notable exceptions being folate, thiamine, vitamins C and K. The 8 micronutrients identified as "problem nutrients" for the pooled group were problematic for each of the age-groups; in addition, median vitamin B6 intake was below the RNI for infants aged 6-8 mo.

Percentage of infants meeting the Recommended Nutrient Intakes

The percentages of infants meeting the RNIs are presented in Table 5 for the non-adjusted data, and for data adjusted for day-to-day variance. By either assumption, the proportion of infants with intakes with adequate intakes was especially low for vitamin D, zinc and iron and low for calcium, pantothenic acid and vitamins K and E. The contribution of breastmilk to the total intake was minimal for vitamin D, iron, vitamin K, zinc, niacin, vitamin B-6 and calcium (in ascending order) and substantial for selenium, vitamin B-12, protein and vitamin A (in descending order).

Observed versus critical nutrient density in complementary feeding

The first data column of Table 6 tabulates nutrient density values in units per 100 kcal for the median intakes of CF observed in the sample; this is disaggregated by age group. Critical nutrient density values using two different models are compared. The first model uses individually-modelled breastmilk intakes observed for the day before the interview, which had median contribution of total energy of 56% for 6-8 mo and 42% for 7-11 mo. Using this variable breastmilk-intake model, 6 vitamins and 3 minerals had observed nutrient density values below the critical nutrient density values for both age-groups. These were the same 8 micronutrients identified as "problem nutrients" in Table 3 (namely, pantothenic acid, vitamins C, D, E and K, calcium, iron and zinc) in addition to vitamin A.

The latter model assumed the WABA-recommended breastmilk-energy contributions of 75% and 50% of energy for the respective age-groups. Furthermore, body weight was assumed as either that of a male infant growing at the 50th percentile of the WHO Child Growth charts[29] or a female infant growing along the 15th percentile. With the fixed breastmilk energy contribution model, the critical nutrient density was higher than the observed nutrient density of CF for 12 of 18 nutrients examined when using the modelling assumption of the worst-case scenario, i.e. a low weight girl aged 6-8 mo. For the best-case scenario, i.e. a boy with a median weight, 5 of 18 nutrients were problematic. In general, however, the lower the breastmilk contribution, the easier it is for the micronutrient content of the observed CF to successfully complement to reach recommended total daily intake.

Vitamins D and K and calcium, iron and zinc had observed nutrient density values below all critical nutrient density values computed, regardless of the modelling used.

Leading food and beverage sources of the "problem nutrients"

Formula milk (infant formulas), whole powdered cow's milk and *Incaparina®*, a commercial fortified grain gruel powder, were each leading sources for 3 of the 9 "*problem nutrients*". Formula milk was amongst the top 3 leading sources of all 9 "*problem nutrients*", it was the main source for vitamins C, E and K, the second most important source for vitamins A and D, calcium and zinc, and the third for pantothenic acid and iron. Whole powdered milk was the main source for pantothenic acid, vitamin D and calcium and third for vitamin C and zinc. Corn, was commonly consumed as a food or drink and an important source of calcium, iron and zinc. Animal sources were mostly limited to dairy products and eggs; beef, although only consumed by 3 infants on the day of interview, was the 9th most important source of zinc. For all "*problem nutrients*" except pantothenic acid and vitamin C, between 2 and 3 sources covered half the daily intakes of each individual nutrient and between 2 and 5 sources covered two-thirds of the intake. The top 10 leading sources of pantothenic acid and vitamin C covered 66% and 63% of the nutrient requirements, respectively. For vitamin D, whole milk powder and formula milk contributed 81% of daily intakes.

DISCUSSION

The urban capital of the Western Highlands of Guatemala has a mixture of ethnic backgrounds and the young children's feeding practices are characterized by almost universal administration of ritual fluids (agüitas) in early infancy,[12] relatively early introduction of CF,[14] and almost universal prolonged partial breast feeding well beyond infancy.[16] With the data obtained from previous-day dietary recalls across the second semester of infancy, we had an opportunity to describe the intake of CF and their nutrient content, and compare the findings with two similar experiences in the central Guatemala.[19,21]

Adequacy of Nutrient Intake and Nutrient Density of Complementary Feeding

There is a 6% higher energy requirement for infants aged 9-11 mo when compared to those aged 6-8 mo. This is accompanied by a 32% higher energy contribution from the CF component of the diet. This is attendant to a 47% lower breastmilk volume and a 33% lower energy contribution from breastmilk (Table 2). Interestingly, as the volume and energy contributions from breastmilk decrease over time, the amounts of vitamins A, C, B12 and pantothenic acid from breastmilk continue to exceed those from CF over the full 6-mo period, whereas the partition for vitamin E and calcium is practically equal across the two sources (Table 3).

Of the 17 nutrients considered in this study, about half were consumed in adequate amounts and the other half were below the RNIs for infants (8 of 17 in pooled sample) (Table 3). Eight micronutrients, 5 vitamins and 3 minerals, were consumed at median intakes below the RNIs throughout the age-range of this study, and only 1 (vitamin B6) was below the RNIs in the diet of the 6-8 mo olds and become adequate in the following 3-mo period (Table 4). With the notable exceptions of vitamins D and K and iron, the percentage of infants satisfying their RNIs generally improved from the 6-8 mo old to the 9-11 mo old period (data not shown). The critical nutrient densities required in the complementary fare are systematically greater when the fixed breastmilk energy contributions of 75% in the third trimester and 50% in the fourth trimester are modelled as compared to those when the actual, variably-modelled contributions are based on assumed individual breastmilk intakes (Table 6).

Comparative Aspects of Complementary Feeding and Total Diet Adequacy in Guatemala

In agreement with our companion reports from two settings in the Central Highlands,[19,21] the magnitude of apparent micronutrient deficits in an urban sample from the Western Highlands is generally less than that reported in other CF studies done in developing countries.[3,5,9,32-37] A diverse array of food and beverage offering, many fortified with vitamins and minerals, may explain the narrowing of the inadequacy gaps across Guatemala. Moreover, the lower energy contribution of breastmilk spares, to a certain extent, the micronutrient-density burden of the CF elements of the diets. The most problematic nutrients continue to mimic those identified by Brown et al[9] in the 1998 report describing several studies and confirmed as problematic across various settings in a more recent review by Osendarp et al,[38] namely iron and zinc.

Limitations of the study

We recognize that our findings are based on data from a relatively small, convenience sample of low-income infants attending the local health clinic in an urban setting. Hence, the representative nature for the population at large may be limited. Nevertheless, overall prevalences of stunting (45%),[15] exclusive breastfeeding rates (8%), and predominant breastfeeding rates (23%) based on dietary recalls since birth (data not shown) were comparable to those reported in the most recent national survey[39] and in smaller studies from our group.[16,19,21] Dietary intakes were recorded in both healthy and ill infants, the latter of which could suppress appetite or alter caretakers' offering of food to the child. Infants visiting the health centre due to illness were not excluded from the analysis because Guatemalan young children spend "normatively" 38% of their life in illness.[40]

Accuracy of maternal reports of infants' previous-day CF intake and the fidelity of food composition tables to reflect actual nutrient content of reported items are intrinsic limiting factors in any recall-based inquiry. Moreover, as the group and sub-group estimates are based on only a previous day dietary recall, true variance was adjusted using NHANES data,[26] which might be different from the expected variability in Guatemala.

The validity of our approach to modelling the energy contribution and volumes of breastmilk may be called into question, but it has been found acceptable in the peer review of previous offerings from our institution.[19-21, 27] The lack of specific data on breastmilk's nutrient composition in Guatemalan mothers, forced us to rely on estimated international values for mature breastmilk in developing countries.[9] The concentration of several micronutrients in breastmilk is affected by maternal deficiency; these are classified as Group I micronutrients and include Thiamin, riboflavin, vitamin B-6, vitamin B-12, and choline.[41] The breastmilk of Guatemalan mothers is likely to have higher amounts of vitamin A and folate due to existing food fortification programs,[42-45] and lower vitamin B12 levels given observed low intakes in this population.[46,47]

Since there are no Estimated Average Requirements (EARs) for infants,[24] we find ourselves obliged to use the RNIs or AIs, which are based on individual requirements, as our standard to assess nutrient inadequacy. Since low-income Guatemalans have less than ideal environmental conditions and poorer maternal welfare, the actual nutritional needs of infants and toddlers in this setting may be higher than the RNIs.[48] There is thus uncertainty in the estimated percentage of infants with inadequate micronutrient intakes in our study population.

A final limitation relates to the true potential of altering the selection of CF towards the foods listed as main sources of "problem nutrients" listed in Appendix 1. Only sources with a nutrient density above the critical nutrient density would improve the diet, whereas those with a lower density would dilute the diet. In our sample, formula milk in a bottle is the main source of vitamins C, E and K, the second leading source of vitamins A and D, calcium and zinc and the third most important source of pantothenic acid and iron. The promotion of formula milk is, however, not advisable as it is likely to displace breastmilk[9] and is a potential source of microbiological contamination.[49,50] In fact, the promotion of any single food item could potentially compromise the overall variety and diversity of the diet, as a given nutrient may not be good sources of other "problem nutrients". In seeking the appropriate blend of CF, however, it should be realized that some sources, such as meat products, may be limited in availability and expensive.[51]

Public health actions to continue to narrow the nutrient gap of mixed-fed infants will come from education in early-feeding practices to select nutrient-rich foods. Available strategies to supply "problem nutrients" include the use of nutrient-dense "indigenous foodstuffs and local foods" as recommended by the WHO[2] or the "optimal use of customary family and indigenous foods rather than focusing solely on fortified foods" as recommended by WABA.[30] Previous modelling by our team, using best-scenario Guatemalan family foods[28] and local infant foods,[27] however, highlight the difficultly of achieving adequate nutrient densities using only local foods. Greater consumption of animal sources could be helpful, but even if accessible to this low-income population, they are unlikely to cover all nutrition requirements for sound infant growth and development. Home fortification, or special fortified foods or a combination, is likely to be required for assuring consumption of the recommended amounts of micronutrients for sound infant growth.

The policy implications of our findings for Guatemalan infants, and likely extending beyond Guatemala to other developing countries, conform to the 1998 WHO/UNICEF recommendation,[9] namely, that home fortification or special fortified foods, or a combination thereof, is likely to be required for assuring consumption of the recommended amounts of micronutrients.

Conclusion

The intake of micronutrients in the second half of the first year of infancy in Guatemala, now documented in the Western Highlands, has a number of notable inadequacies, but the gaps are narrower than traditionally reported for this age group in low-income settings. The interplay between breastmilk intake and adequate CF is the determinant basis for achieving recommended daily intakes.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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Table 1. Demographic characteristics of the infants and mothers

	Entire sample(n=94)	
	Number	Proportion (%)
Gender		
Boys	44	47
Girls	50	53
Residential area		
Urban (*Quetzaltenango)*	50	53
Semi-urban (*La Esperanza)*	44	47
Age categories		
6-8 mo	52	55
9-11 mo	42	45
Place of birth		
National hospital	62	66
Private clinic/hospital	16	17
Home setting	16	17
Teenage mother (aged ≤19 y)		
No	77	82
Yes	16	17
Marital status mother		
Single	15	16
United or married	76	81
Divorced or widowed	3	3
Number of children		
1 child	37	39
>1 child	57	61
Level of education mother		
Primary schooling or less	58	62
Secondary schooling or higher	36	38
Occupation of the mother		
Housewife or working at home	72	77
Works outside the home	22	23
Ethnicity[†]		
Ladina	51	54
Indigenous	43	46

[†]Ethnicity was based on observation of clothing worn on the day of the interview. Indigenous women traditionally wear traditional native clothing (huipiles and cortes). Ladinas, officially recognized as a distinct ethnic group, dresses in a style commonly considered as western.

Table 2. Daily energy requirements, energy contribution from complementary feeding and modelled energy contribution from breastmilk

	Age-groups							
	6-8 mo (n=52)				9-11 mo (n=42)			
	Mean±SD	Median	Min	Max	Mean±SD	Median	Min	Max
Calculated energy requirement (kcal)†	552±81	561	400	733	605±96	594	396	917
Estimated energy intakes from complementary feeding (kcal)‡	273±201	237	12	815	364±198	351	9	920
Modelled energy intakes from breastmilk (kcal)§	288±180	331	0††	620	259±192	224	0††	748
Modelled volume of breastmilk consumed (ml)¶	430±268	493	0††	926	387±287	335	0††	1117
Energy contribution from breastmilk to total intake (%)	52±32	56	0††	98	41±28	42	0††	99

†Energy requirements were computed using the formula: -95.4 + 88.3 * measured body weight (kg).[22]
‡Estimated daily intake based on a single previous-day dietary recall.
§Computed as the difference between energy requirement and estimated energy intakes from complementary feeding.
¶Computed from the energy contribution of breastmilk assuming that the energy content of breastmilk is 67 kcal/100 mL.[9]
††When the estimated energy from complementary foods was higher than the energy requirement, the volume of breastmilk was artificially set at zero.

Table 3. Daily energy requirements, energy contribution from complementary feeding and modelled energy contribution from breastmilk

	Requirement	Daily intake from complementary feeding[¶]		Daily intake from breastmilk[††]		Daily intake from complementary feeding and breastmilk	
		Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
Water (g)	800[†]	334±217	305	358±242	356	692±183	672
Protein (g)	10/11[‡]	11±9	8	4±3	4	15±8	13
	RNI[§]						
Vitamins							
Folate DFE (µg)	80	70±52	64	35±24	35	105±41	104
Pantothenic acid (mg)	1.8	0.86±0.84	0.61	0.74±0.50	0.73	1.59±0.69	1.45*
Thiamin (mg)	0.3	0.46±0.47	0.30	0.09±0.06	0.09	0.55±0.44	0.38
Riboflavin (mg)	0.4	0.48±0.46	0.33	0.14±0.10	0.14	0.62±0.40	0.50
Niacin (mg)	4.0	6.1±6.0	4.4	0.6±0.4	0.6	6.8±5.8	4.6
Vitamin B-6 (mg)	0.3	0.34±0.27	0.25	0.04±0.03	0.04	0.38±0.26	0.30
Vitamin B-12 (µg)	0.7	0.84±2.39	0.33	0.40±0.27	0.39	1.24±2.36	0.81
Vitamin C (mg)	30	14±16	8	16±11	16	30±16	27*
Vitamin A (RAE)	400	347±500	162	205±138	203	552±469	445
Vitamin D (µg)	5.0	1.3±2.3	0.3	0.2±0.2	0.2	1.5±2.2	0.7*
Vitamin E (mg)	2.7	1.5±1.6	1.0	0.9±0.6	0.9	2.5±1.4	2.1*
Vitamin K (µg)	10.0	7.4±8.7	4.0	0.9±0.6	0.9	8.3±8.5	4.9*
Minerals							
Calcium (mg)	400	221±241	127	115±77	114	335±202	265*
Iron (mg)	6.2/18.6	6.2±7.4	3.1	0.1±0.1	0.1	6.3±7.3	3.2*
Magnesium (mg)	54.0	67.1±53.7	57.8	14.3±9.7	14.2	81.5±47.1	72.3
Selenium (µg)	10.0	14.9±12.8	13.2	8.2±5.5	8.1	23.1±10.1	20.6
Zinc (mg)	0.8/8.4	3.0±3.7	1.9	0.5±0.3	0.5	3.5±3.5	2.4*

AI: Adequate Intake; RAE: Retinol Activity Equivalents; RNIs: Reference Nutrient Intake; DFE: Dietary Folate Equivalent.
[†]Adequate Intake (AI) for total water.[31]
[‡]Based on the safe level of protein intake for infants of 1.3 g/kg.[23]
[§]RNIs values for infants aged 7-12 mo old.[24]
[¶]Based on a single previous-day recall in 94 infants aged 6-11 mo.
[††]Breastmilk intakes were modelled based energy requirements computed using the formula: -95.4 + 88.3* measured body weight (kg).[22]
*Estimated median intake below the RNI.

Table 4. Estimated 24-h intakes of water, protein and 17 selected micronutrients from complementary feeding and modelled volumes of breastmilk combined, disaggregated by age-groups (6-8 and 9-11 mo)

	Requirement	Daily intake from complementary feeding and breastmilk[¶]			
		6-8 mo (n=52)		9-11 mo (n=42)	
		Mean±SD	Median	Mean±SD	Median
Water (g)	800[†]	662±171	658	727±191	688
Protein (g)	10/11[‡]	13±6	11	16±9	14
	RNI[§]				
Vitamins					
Folate DFE (µg)	80	104±42	104	106±40	104
Pantothenic acid (mg)	1.8	1.52±0.55	1.42*	1.68±0.82	1.49*
Thiamin (mg)	0.3	0.50±0.32	0.41	0.61±0.54	0.35
Riboflavin (mg)	0.4	0.57±0.35	0.43	0.68±0.44	0.53
Niacin (mg)	4.0	5.9±4.3	4.3	7.8±7.1	5.5
Vitamin B-6 (mg)	0.3	0.34±0.23	0.26*	0.42±0.29	0.33
Vitamin B-12 (µg)	0.7	0.90±0.61	0.79	1.62±3.36	0.99
Vitamin C (mg)	30	32±15	29*	28±16	25*
Vitamin A (RAE)	400	497±331	441	614±586	455
Vitamin D (µg)	5.0	1.5±2.3	0.5*	1.5±2.0	0.8*
Vitamin E (mg)	2.7	2.4±1.4	1.9*	2.6±1.4	2.2*
Vitamin K (µg)	10.0	9.1±9.9	5.2*	7.4±6.6	4.9*
Minerals					
Calcium (mg)	400	316±186	253*	358±219	282*
Iron (mg)	6.2/18.6	5.5±5.9	3.1*	7.2±8.7	3.2*
Magnesium (mg)	54.0	72.1±37.5	63.2	92.1±54.5	76.0
Selenium (µg)	10.0	21.1±9.0	19.2	25.4±10.9	22.9
Zinc (mg)	0.8/8.4	2.9±2.3	2.3*	4.2±4.5	2.7*

AI: Adequate Intake; RAE: Retinol Activity Equivalents; RNIs: Reference Nutrient Intake; DFE: Dietary Folate Equivalent.
[†]Adequate Intake (AI) for total water.[31]
[‡]Based on the safe level of protein intake for infants of 1.3 g/kg.[23]
[§]RNIs values for infants aged 7-12 mo old.[24]
[¶]Based on a single previous-day recall in 94 infants aged 6-11 mo.
[††]Breastmilk intakes were modelled based energy requirements computed using the formula: -95.4 + 88.3 * measured body weight (kg).[22]
*Estimated median intake below the RNI.

Table 5. Percentage of infants meeting the Reference Nutrient Intake (RNIs) for selected nutrients, based on complementary feeding and breastmilk combined †

	Percentage of infants meeting the RNIs‡ (%)	
	Non-adjusted data§	Variance adjusted data§
Protein	78	93
Vitamins		
Folate DFE	69	85
Pantothenic acid	27	25
Thiamin	70	91
Riboflavin	63	87
Niacin	56	81
Vitamin B-6	49	66
Vitamin B-12	66	81
Vitamin C	45	46
Vitamin A	59	71
Vitamin D	5	3
Vitamin E	31	33
Vitamin K	27	29
Minerals		
Calcium	26	22
Iron (mg)¶	10	-
Magnesium	65	87
Selenium	100	100
Zinc¶	7	3

RAE: Retinol Activity Equivalents; RNIs: Reference Nutrient Intake; DFE: Dietary Folate Equivalent.

†Based on a single previous-day recall in 94 infants aged 6-11 mo and modelled breastmilk intakes based on age- and gender-specific energy requirements.[22]

‡RNIs values for infants aged 7-12 mo old.[24]

§Since dietary estimates were based on a single previous-day recall, estimated distributions of usual intake of nutrients were adjusted for day-to-day variance using "Intake Modeling, Assessment and Planning Program" (IMAPP).[25] This software uses the variance in the diet using data from the National Health and Nutrition Examination Survey (NHANES)[26] and assumes similar variance in other populations.

¶Assuming low bioavailability.

Table 6. Observed protein and micronutrient density of complementary feeding based on a single previous-day recall in relation to critical nutrient densities† modelled with variable versus fixed breastmilk energy contribution, disaggregated by age-groups (6-8 and 9-11 mo)

	Median nutrient density as nutrient unit per 100 kcal		
	Observed nutrient density of complementary feeding‡	Critical nutrient density	
		Distributional variable breastmilk -intake model§	Fixed breastmilk energy contribution model¶
6–8 mo	(n=52)	(n=52)	Modelled
Protein (g)	2.8	2.0	1.9–2.0
Vitamins			
Folate DFE (µg)	21.6	16.0	9.0–20.5
Pantothenic acid (mg)	0.25	0.38*	0.25–0.51*
Thiamin (mg)	0.13	0.09	0.08 – 0.13
Riboflavin (mg)	0.11	0.09	0.08 – 0.14*
Niacin (mg)	1.6	1.4	1.7*–2.3*
Vitamin B-6 (mg)	0.11	0.11	0.13*–0.18*
Vitamin B-12 (µg)	0.11	0.10	0–0.08
Vitamin C (mg)	4.1	4.6*	0–4.1
Vitamin A (RAE)	61.7	66.1*	11.5–69.0*
Vitamin D (µg)	0.0	2.0*	2.7*–3.4*
Vitamin E (mg)	0.38	0.66*	0.56*–0.95*
Vitamin K (µg)	1.8	3.8*	4.9*–6.4*
Minerals			
Calcium (mg)	55.0	113.4*	110.0*–167*
Iron (mg)[d]	1.4	7.8*	10.8*–13.4*
Magnesium (mg)	20.7	15.8	16.1–23.8*
Selenium (µg)	3.6	0.1	0
Zinc (mg)[d]	0.6	3.3*	4.4*–5.6*
9–11 mo	(n=42)	(n=42)	Modelled
Protein (g)	3.2	1.7	1.7–1.8
Vitamin s			
Folate DFE (µg)	22.3	13.4	8.6–13.6
Pantothenic acid (mg)	0.24	0.31*	0.21–0.32*
Thiamin (mg)	0.09	0.06	0.05–0.07
Riboflavin (mg)	0.12	0.08	0.05–0.08
Niacin (mg)	1.4	1.0	0.8–1.1
Vitamin B-6 (mg)	0.10	0.07	0.07 – 0.08
Vitamin B-12 (µg)	0.18	0.10	0.04–0.1
Vitamin C (mg)	2.4	4.3*	2.0–3.9*
Vitamin A (RAE)	60.0	63.2 *	31.8–56.9
Vitamin D (µg)	0.2	1.4*	1.2*–1.6*
Vitamin E (mg)	0.39	0.53*	0.37–0.54*
Vitamin K (µg)	1.4	2.6*	2.3*–3.0*
Minerals			
Calcium (mg)	41.9	83.4*	64.6*–89.8*
Iron (mg)¶¶	1.2	5.3*	4.9*–6.1*
Magnesium (mg)	17.4	11.6	9.1–12.5
Selenium (µg)	4.4	0.9	0–0.3
Zinc (mg)¶¶	0.7	2.2*	2.1*–2.6*

RAE: Retinol Activity Equivalents; DFE: Dietary Folate Equivalent.

†The critical nutrient density of CF is the nutrient level that would complement the assumed breastmilk consumption and can be used as a reference of adequate intakes.

‡Observed intakes based on a single previous-day recall in 94 infants aged 6-11 mo;

§Critical nutrient density for complementary feeding calculated as the difference between the daily RNI values and the nutrient. content of individually modelled volumes of breastmilk, divided by the energy content of complementary feeding assessed by means of a single previous-day dietary recall in 94 infants.

¶Critical nutrient density for complementary feeding, the lowest value is for a boy following the 50th WHO growth percentile[29] and the highest values is for a girl following the 15th WHO growth percentile as published previously by our group assuming energy contribution from complementary feeding to be 25% in the 7th-9th month of age and 50% in the 10th-12th month.[27,28]

¶¶Assuming low bioavailability.

*The critical nutrient density is higher than the observed nutrient density of complementary feeding.

Appendix 1. The ten leading food sources of 9 individual “problem nutrients” in complementary feeding[†]

Rank	Item	%[‡]	Cum %[§]	Item	%[‡]	Cum %[§]	Item	%[‡]	Cum %[§]
	Pantothenic Acid			**Vitamin C**			**Vitamin A**		
1	Whole milk, powder	21	21	Formula milk	34	34	*Incaparina®* [¶]	35	35
2	Scrambled eggs	8	29	Packaged juice	4	38	Formula milk	16	51
3	Formula milk	7	36	Whole milk, powder	4	42	Carrots	10	61
4	*Incaparina®* [¶]	7	43	Banana	4	46	Whole milk, powder	7	68
5	Boiled eggs	7	50	Potatoes	4	50	*Corazon de trigo®* [¶]	4	72
6	Potatoes	5	55	Papaya	4	53	Scrambled eggs	3	76
7	Banana	3	58	Artificial fruit drink, powder	3	57	Boiled eggs	2	78
8	Corn *Tamalito*	3	61	Chilli pepper	2	59	Corn flakes	2	80
9	White rice	3	64	Apple compote	2	61	Rice soup	2	82
10	Black tea	2	66	Strawberries	2	63	Liver	1	83
	Vitamin D			**Vitamin E**			**Vitamin K**		
1	Whole milk, powder	42	42	Formula milk	43	43	Formula milk	48	48
2	Formula milk	38	81	*Nestum®* [d]	8	52	Carrots	5	54
3	Scrambled eggs	5	86	Oil	6	58	Corn *Tamalito*	5	58
4	Boiled eggs	5	91	Noodle soup	5	63	Broccoli	4	63
5	Whole milk, fluid	3	94	Scrambled eggs	5	67	Rice soup	3	66
6	Corn flakes	2	96	Whole milk, powder	3	71	Scrambled eggs	3	69
7	Fried eggs	1	97	Boiled eggs	3	74	Plantain, fried	3	71
8	Soya milk, powder	1	98	Carrots	2	76	Sweet rolls	2	74
9	Pediasure®	0	99	*Incaparina®* [¶]	1	78	Potatoes	2	76
10	Fish	0	99	Vegetable soup	1	79	Black bean soup	2	78
	Calcium			**Iron**			**Zinc**		
1	Whole milk, powder	30	30	*Incaparina®* [¶]	22	22	*Incaparina®* [¶]	38	38
2	Formula milk	17	47	*Nestum®* [¶]	20	42	Formula milk	14	52
3	*Incaparina®* [¶]	14	60	Formula milk	16	58	Whole milk, powder	8	59
4	*Nestum®* [¶]	8	69	Corn flakes	6	64	*Corazon de trigo®* [¶]	4	64
5	Maize tortilla	4	72	Sweet rolls	4	68	Noodle soup	2	66
6	Sweet rolls	3	75	*Corazon de trigo®* [¶]	3	70	Corn *Tamalito*	2	68
7	Water	3	78	Corn drink	2	72	Scrambled eggs	2	70
8	Corn *Tamalito*	2	80	Corn *Tamalito*	2	74	Oatmeal	2	72
9	Whole milk, fluid	2	82	Corn flour	2	76	Beef, shredded	1	73
10	Scrambled eggs	2	84	Oatmeal	2	77	*Nestum®* [¶]	1	74

[†]Based on a single previous-day recall in 94 infants aged 6-11 mo.
[‡]Percent contribution.
[§]Cumulative percent contribution.
[¶]Fortified cereals.