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Developing lipid-based nutrient supplements

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Original Article

Considerations in developing lipid-based nutrient supplements for prevention of undernutrition: experience from the International Lipid-Based Nutrient Supplements (iLiNS) Project

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Abstract

The International Lipid-Based Nutrient Supplements (iLiNS) Project began in 2009 with the goal of contributing to the evidence base regarding the potential of lipid-based nutrient supplements (LNS) to prevent undernutrition in vulnerable populations. The first project objective was the development of acceptable LNS products for infants 6–24 months and for pregnant and lactating women, for use in studies in three countries (Burkina Faso, Ghana and Malawi). This paper shares the rationale for a series of decisions in supplement formulation and design, including those related to ration size, ingredients, nutrient content, safety and quality, and packaging. Most iLiNS supplements have a daily ration size of 20 g and are intended for home fortification of local diets. For infants, this ration size is designed to avoid displacement of breast milk and to allow for dietary diversity including any locally available and accessible nutrient-dense foods. Selection of ingredients depends on acceptability of flavour, micronutrient, anti-nutrient and essential fatty acid contents. The nutrient content of LNS designed to prevent undernutrition reflects the likelihood that in many resource-poor settings, diets of the most nutritionally vulnerable individuals (infants, young children, and pregnant and lactating women) are likely to be deficient in multiple micronutrients and, possibly, in essential fatty acids. During ingredient procurement and LNS production, safety and quality control procedures are required to prevent contamination with toxins or pathogens and to ensure that the product remains stable and palatable over time. Packaging design decisions must include consideration of product protection, stability, convenience and portion control.

Keywords: home fortification, undernutrition, prevention, infant, young child, pregnancy, lactation, micronutrients, essential fatty acids, Africa.

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Introduction

Undernutrition is an unacceptably common problem among infants and young children and women of child-bearing age in many resource-poor settings. The consequences of undernutrition among infants and young children can be severe both for individuals and for societies, and include increased risks of morbidity and mortality, delayed or compromised motor and cognitive development, decreased school achievement, increased risk of chronic diseases later in life and reduced economic productivity (World Bank 2006). Recognition of both the human and the economic costs has recently led to a renewed global

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consensus and commitment around intervening to prevent undernutrition during the 'first 1000 days', from conception up to age 2 years. Prevention is emphasised because some of the damage done during the first 1000 days is irreversible (Victora *et al*. 2008).

Undernutrition – stunting, wasting and micronutrient deficiencies – has many causes and requires commitment and action from multiple sectors. One important dimension relates to the adequacy of infant and young-child feeding. Promotion, protection and support of optimal breastfeeding are critical public health actions, and programming around breastfeeding has a long history (Piwoz *et al*. 2003). Complementary feeding – that is, feeding infants during the transition from exclusive breastfeeding until they are fully weaned – also represents a critical challenge, as infants between 6 and 23 months of age (the usual period of complementary feeding) require nutrient-dense food (Dewey & Brown 2003). Unfortunately, available and affordable foods often fail to meet nutrient needs, particularly when families cannot afford frequent consumption of animal-source foods, such as meat, fish, eggs and dairy products (Dewey & Brown 2003; Vitta & Dewey 2012). Women similarly face challenges in meeting their higher nutrient needs during pregnancy and lactation (Torheim *et al*. 2010; Lee *et al*. 2012).

A variety of integrated approaches is required to overcome these challenges, including encouragement of dietary diversification and provision of supplements and/or fortified food products, which may be provided along with food aid and/or subsidised in some situations. 'Home fortification' may play a special role as it represents one strategy for targeted delivery of specific nutrients to individuals with high requirements for nutrient density (women of reproductive age, infants and young children). Two kinds of 'home fortificants' have been used to date: micronutrient powders (MNP),[1] which are efficacious in reducing anaemia and iron deficiency (De-Regil *et al*. 2011), and small-quantity lipid-based nutrient supplements (LNS), which are currently under study. Both MNP and LNS are easy for infants to consume (unlike multiple micronutrient supplements in pill form). LNS differ from MNP because they are produced in a food base and deliver energy, protein and essential fatty acids (EFA), as well as a wider range of micronutrients than MNP, including the macrominerals required for growth. Up until now, interventions providing MNP have not increased linear growth (De-Regil *et al*. 2011). This suggests either that micronutrients alone may not be sufficient to stimulate linear growth or that the range or amount of

[1]ALA, alpha-linolenic acid; a_w, water activity; CoA, certificates of analyses; CCNFSDU, Codex Committee on Nutrition and Foods for Special Dietary Uses; CSB, corn-soy blend; DHA, docosahexaenoic acid; DRI, United States–Canada Dietary Reference Intake(s); DSM, dried skim milk; EFA, essential fatty acid(s); FBF, fortified blended food(s); HACCP, Hazard Analysis Critical Control Points; iLiNS Project, International Lipid-based Nutrient Supplements Project; IOM, Institute of Medicine; ISO, International Organization for Standardization; LA, linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acid; LNS, lipid-based nutrient supplement(s); MAM, moderate acute malnutrition; MNP, micronutrient powder(s); ppb, parts per billion; RUSF, ready-to-use supplementary food(s); RUTF, ready-to-use therapeutic food(s); SAM, severe acute malnutrition; UNIMMAP, United Nations International Multiple Micronutrient Preparation.

Key messages

- Small-quantity lipid-based nutrient supplements (SQ-LNS, at ~20 g or ~110–120 kcal day^{-1}) are designed to enrich infant and maternal diets, as one type of home fortification of local foods.
- For infants, the ration size is designed to avoid displacement of breast milk and allow for dietary diversity.
- SQ-LNS are designed to support adequate maternal nutritional status and infant growth and development in settings where diets are likely to be low in multiple nutrients, including macrominerals, other micronutrients and essential fatty acids.
- Considerations in developing supplements for prevention of undernutrition include the ingredients, ration size, nutrient content, safety and quality concerns, and packaging.

micronutrients provided (to date) in MNP has not been sufficient (Piwoz *et al*. 2012).

Presently, there is interest in LNS for a variety of applications and cultural/geographical settings. Many organisations and companies are engaged in new product formulation. Researchers in our consortium, the International Lipid-Based Nutrient Supplements (iLiNS) Project (http://www.iLiNS.org), are currently engaged in a series of efficacy trials intended to contribute to an evidence base defining the potential of LNS to play a role in prevention of undernutrition.

The objective of this document is to share the rationale for a range of decisions made in developing iLiNS Project supplements. Most of these decisions related to the content of the supplement itself (ingredients, nutrients and quality concerns), but we also discuss packaging options.

The project is a collaboration among many partners, including researchers from the University of California, Davis, USA; the Institut de Recherche en Sciences de la Santé, Burkina Faso; the University of Ghana; the University of Malawi College of Medicine; the University of Tampere, Finland; Nutriset; Project Peanut Butter, Malawi; the US Department of Agriculture (USDA), ARS Western Human Nutrition Research Center; and Helen Keller International. Many of these collaborating researchers, who are acknowledged at the end of this paper, contributed to the rationale and decisions about iLiNS Project supplements described herein. The iLiNS Project was initiated in 2009 with the following goal and objectives:

iLiNS Project goal

The goal of the project is to contribute to the evidence base regarding the use of LNS to prevent undernutrition in vulnerable populations.

iLiNS Project objectives

1. Develop and test the acceptability of alternative LNS formulations for various target groups;
2. Evaluate the efficacy of reduced cost formulations of LNS for infants and young children;
3. Determine the optimal amount of zinc to include in LNS;
4. Evaluate the efficacy of LNS products for pregnant and lactating women;
5. Conduct socio-economic studies of LNS (demand, delivery systems and cost-effectiveness); and
6. Coordinate efforts, build capacity and communicate results to inform policies and programmes.

This document presents early lessons learned under objective 1, now completed. In addition to this document, which describes considerations for formulating and packaging LNS for prevention, our results related to short-term acceptability of the iLiNS supplements are available (Adu-Afarwuah *et al*. 2011; Hess *et al*. 2011; Phuka *et al*. 2011). In our three project sites within Burkina Faso, Ghana and Malawi, the iLiNS supplements were found to be highly acceptable to both mothers and infants in short-term trials. Acceptability was assessed based on direct observation of consumption by infants in one feeding and disappearance of the supplement in a 2-week trial, the mother's own liking of the supplement (a hedonic scale), and her perceptions of the infant's acceptance and ease of feeding. Further information on acceptability and adherence is being gathered in longer-term clinical trials and associated socio-economic studies, currently ongoing (objectives 2–5 above).

In the following sections, we present background information and the rationale for choices made for iLiNS supplements in relation to:

- ingredients;
- energy dose per day;
- micronutrient and EFA content;
- safety and quality; and
- packaging.

Before proceeding to these topics, it is useful to review the range of lipid-based products used to date in treatment and prevention of undernutrition (Box 1).

Ready-to-use therapeutic foods

Ready-to-use therapeutic foods (RUTF) are now used widely to treat severe acute malnutrition (SAM), primarily in outpatient programmes. RUTF

Box 1. Classification of lipid-based products

Ready-to-use therapeutic foods/large-quantity LNS
- Designed for treatment of severe acute malnutrition
- Provide 100% of energy needed from foods other than breast milk
- Typical ration of ~180–280 g day^{-1} (~1000–1500 kcal, or 200 kcal kg^{-1} day^{-1})

Ready-to-use supplementary foods/medium-quantity LNS
- Designed for treatment of moderate acute malnutrition
- Also used for prevention of seasonal wasting/prevention of undernutrition in food-insecure populations
- Provide 50–100% of energy needed from foods other than breast milk
- Typical ration of ~45–90 g day^{-1} (~250–500 kcal day^{-1})

LNS for home fortification/small-quantity LNS
- Designed to prevent undernutrition and promote growth and development
- Provide <50% of energy needed from foods other than breast milk[a]
- Typical ration of ~20 g day^{-1} (~110 kcal day^{-1})

Note: All LNS contain most or all of the daily micronutrient requirements; hence, micronutrient density increases progressively as the daily ration size decreases.
[a]Slightly higher than 50% for breastfed infants 6–8 months of age.

were designed to achieve accelerated daily weight gains to reach a target weight-for-height that is consistent with nutritional recovery. Thus, RUTF temporarily replace most or all foods other than breast milk. There is growing evidence that programmes using RUTF result in better outcomes and fewer deaths compared with the previous standard of care (Diop *et al*. 2003; Ciliberto *et al*. 2005). RUTF are provided in large quantities, typically around 200 kcal kg^{-1} day^{-1} during a 6–8-week period of rehabilitation (World Health Organization/World Food Program/Standing Committee on Nutrition/United Nations Children's Fund 2007).

One randomised trial reported positive results for use of RUTF (1000 kcal day^{-1}) for treatment of children with moderate acute malnutrition (MAM), compared with the provision of corn-soy blend, oil and sugar (CSB, 1231 kcal day^{-1}) (Nackers *et al*. 2010). One cluster-randomised trial reported positive results from use of RUTF to prevent seasonal wasting with a 3-month ration of 500 kcal day^{-1} (92 g) compared with a null control (Isanaka *et al*. 2009); note that this use of RUTF was in a lower dosage than the therapeutic (treatment) dose.

Ready-to-use supplementary foods/medium-quantity LNS

Several other products, designed to be consumed in smaller quantities and often described as ready-to-use supplementary foods (RUSF), have been used for treatment of MAM (Matilsky *et al*. 2009; Lagrone *et al*. 2010; Ackatia-Armah *et al*. 2012; LaGrone *et al*. 2012) and for preventing seasonal increases in acute malnutrition (Isanaka *et al*. 2010; Huybregts *et al*. 2012; Talley *et al*. 2012). Dosages used for treating MAM range from 65 to 75 kcal kg^{-1} day^{-1}, while dosages for prevention of seasonal wasting typically are ~250 kcal (or 45–50 g) day^{-1}. The term 'RUSF' is currently inconsistently used, with some suggesting it be used only to refer to products designed for treatment of MAM (Global Nutrition Cluster 2012) and others using the term to cover medium-quantity products designed both for treatment and for prevention of wasting (Isanaka *et al*. 2010; Grellety *et al*. 2012; Huybregts *et al*. 2012).

In contrast to RUTF, for which evidence for improved recovery is strong, evidence to date for use of RUSF to treat MAM or prevent wasting is mixed and has not established clear superiority compared with alternatives (Huybregts *et al*. 2012; LaGrone *et al*. 2012). Additional studies are ongoing.

Medium-quantity LNS has also been used in a series of studies in Malawi, and one in the DRC, aiming to prevent stunting and/or underweight or to increase growth of underweight infants (Kuusipalo *et al*. 2006; Lin *et al*. 2008; Phuka *et al*. 2008, 2009a,b; Thakwalakwa *et al*. 2010, 2012; Bisimwa *et al*. 2012). Results of these studies have also been mixed and may reflect differences in baseline nutritional status, prevalence of underlying infections (e.g. HIV), study foods and comparison foods, and duration of intervention (Dewey & Arimond 2012).

LNS for home fortification/small-quantity LNS

LNS products with a lower energy dose (~110 kcal-day^{-1}) but a full complement of micronutrients have

been shown to prevent faltering in linear growth from 6 to 11 months and to support normal motor development in Ghana (Adu-Afarwuah *et al*. 2007b) and to partially prevent severe stunting in Malawi (Phuka *et al*. 2008). The iLiNS Project involves researchers from these trials and aims to re-examine and extend the previous study results using modified formulations of the supplements. As with RUSF/medium-quantity LNS, the evidence base for small-quantity LNS used to enrich home diets is still limited and evolving.

Considerations for selecting ingredients

LNS recipes can include a variety of ingredients but typically have included vegetable oil, peanut/groundnut paste, milk powder and sugar. Alternative recipes and formulations are currently being explored in efforts to develop lower cost and culturally acceptable products for a range of settings, often based on locally produced foods. Other ingredients have included whey, soy protein isolate, and sesame, cashew and chickpea paste, among others. Flavouring ingredients have included tomato, cumin, onion, cardamom or cinnamon; and producers continue to experiment with new flavours.

Considerations in selecting food ingredients for an LNS formulation (Box 2) include:

- cost;
- acceptability of flavours;
- fat content, and content and balance of EFA;
- current knowledge about the role of milk in infant/young child growth;
- selection of legumes/nuts and use of grains;
- sugar content; and
- anti-nutrients.

iLiNS Project decisions on ingredients were also influenced by the products previously used by the research teams in Ghana and Malawi (Adu-Afarwuah *et al*. 2007b, 2008; Phuka *et al*. 2008, 2009b). The ingredients in the iLiNS supplements, in order by amount, are soybean oil, dried skim milk (DSM), peanut, sugar, a vitamin/mineral premix, maltodextrin (modified starch), stabilisers and emulsifiers. The order stated is for the 20-g formulation with milk. Several different formulations are being tested (different daily doses, and with and without milk), and the order (relative amount) of ingredients varies slightly among formulations.

Box 2. Key considerations for ingredients in LNS

- iLiNS supplement recipes evolved from earlier successful trials; most iLiNS supplements contain soybean oil, dried skim milk, peanut, sugar, a vitamin/mineral premix, maltodextrin (modified starch), stabilisers and emulsifiers (two trial supplements include no milk).
- iLiNS supplements contain essential fatty acids, especially the omega-3 fatty acid alpha-linolenic acid; levels are higher than in a similar LNS developed previously (Nutributter®) (Table 2).
- Milk may promote growth in unique ways, but the exact mechanism is unknown; it is also not known if LNS with a small amount of milk prevent stunting and poor development better than LNS with no milk. One iLiNS study is designed to answer this question.
- Peanut-flavoured LNS are very acceptable in our study areas and many others, but many different flavours of LNS are possible.
- Including low-fat grains and legumes in LNS may necessitate a higher overall energy dose to reach the target for essential fatty acid content; this increases risk of displacing breast milk and other foods.

Cost

Cost was considered in two ways during formulation of iLiNS supplements. Because milk costs have fluctuated over time and were thought to potentially represent a high proportion of ingredient costs, one iLiNS study includes a comparison to supplements with no DSM. The same study is also exploring the impact of different quantities of food base, as this substantially affects cost. Other than in relation to milk, cost was not considered in selection of ingredients because, as noted, the supplements evolved from earlier research products that had shown some positive results.

Acceptability of flavour

In earlier studies with RUTF, supplements formulated with peanut as a main ingredient have been well

accepted by infants and their mothers. Most of these interventions and studies have been in Africa. This may be in part because in many African settings, peanuts (usually called groundnuts) are common and familiar in local diets.

In earlier work in two of our three study sites (Ghana and Malawi), peanut-flavoured LNS (20–50 g range for daily dose) was well accepted. Therefore, peanut was selected as a main food ingredient and flavour in our project supplements.

To date, the acceptability of LNS with peanut as a main ingredient has also been confirmed in a number of trials in Africa, including in Burkina Faso, Ghana, Malawi, Niger, Uganda and Zimbabwe (Flax *et al*. 2009; Adu-Afarwuah *et al*. 2011; Hess *et al*. 2011; Parker *et al*. 2011; Phuka *et al*. 2011; Tripp *et al*. 2011; Cohuet *et al*. 2012; Ickes *et al*. 2012; Paul *et al*. 2012). Peanut-flavoured LNS have also been found acceptable in other regions [Haiti (Heidkamp *et al*. 2012); Guatemala (Matias *et al*. 2011); and Bangladesh (Mridha *et al*. 2012)]. Most acceptability studies have been short-term (from 12 days to 12 weeks); a few authors have reported on usage and acceptability after somewhat longer programmatic interventions (6 months). iLiNS researchers continue to assess acceptability over the longer term in the ongoing efficacy trials. In addition to acceptability of flavour, iLiNS researchers are assessing overall acceptability of long-term daily use of the LNS by infants and pregnant and lactating women.

Fat content, and content and balance of essential fatty acids

LNS are so named ('lipid-based') because lipids (fats) provide the majority of the energy content. Several considerations determined the fat content of the iLiNS supplements.

The first is relevant to all LNS and relates to food safety. The technology used to manufacture LNS (used by Nutriset and the Plumpy'Field Network) involves embedding micronutrients in a fat-based matrix. This results in low water activity ($a_w < 0.5$) in the products. Low water activity prevents the growth of bacteria, yeast and fungi, and theoretically reduces non-enzymatic browning and lipid oxidation (Labuza *et al*. 1977).

Box 3. Essential Fatty Acids

Like many vitamins and minerals, EFA cannot be produced by the human body and must be provided in diets (hence they are 'essential'). There are two EFA: the omega-3 fatty acid alpha-linolenic acid (ALA) and the omega-6 fatty acid linoleic acid (LA). While the functions of EFA and their derivatives are not as well understood as those of vitamins and minerals, EFA are known to play a number of critical structural and functional roles in the human body, including in the brain (Innis 2008; Lauritzen & Carlson 2011; Makrides *et al*. 2011).

EFA are present in animal-source foods and in certain plant-source oils. Diets are likely to be low in EFA, especially ALA, when they include few animal-source foods and are low in total fat, or the fat sources are poor sources of EFA (Huffman *et al*. 2011; Michaelsen *et al*. 2011). Thus, LNS are designed to be good sources of EFA, in addition to vitamins and minerals.

iLiNS supplements have a particularly high lipid content for an additional reason. Path analysis of data from the earlier trial in Ghana suggested that EFA (see Box 3) and, particularly, the omega-3 fatty acid alpha-linolenic acid (ALA), played a role in promoting linear growth because the increase in plasma ALA concentration observed in the LNS group explained (statistically) a large proportion of the greater length gain observed in that group, compared with the groups receiving micronutrients only (Adu-Afarwuah *et al*. 2007a). iLiNS collaborators therefore chose to prioritise the ALA content of the study supplements. During product development, Nutriset aimed to maximise ALA content while maintaining stability and producing a palatable product.

Plant-source oils may or may not provide adequate amounts of key EFA. Oil/seeds that contain the largest amounts of ALA include flaxseed, walnut, beechnut, butternut, chia seeds, canola and soy. Oils such as corn, sunflower, palm and peanut oil are high in LA but low in ALA (Huffman *et al*. 2011). The most common of the high-ALA oils noted earlier are canola and soy, and these may be good choices for LNS formulations.

In some cases, where projects or producers aim to source or produce LNS close to the areas where it will be consumed, local availability (and price) of oils will also be a consideration in selecting oils as ingredients.

For our research purposes, iLiNS Project supplements are produced by Nutriset in France and contain soybean oil in addition to the oil provided by peanut. Using these oils, it was possible to meet targets for EFA content in the overall supplement formulations. Soybean oil was selected in preference to canola oil because the latter is not widely available in our study countries; this keeps open the possibility of future local production of the tested formulations.

The final issue in relation to fat content in LNS is the issue of product stability, particularly the potential for oxidation reactions. Products with high concentrations of fat and certain micronutrients with oxidative capacity (e.g. iron) are particularly vulnerable to oxidation and development of rancid flavours. This presents technical problems that were overcome by Nutriset during iterative work formulating the iLiNS supplements. The technical problems were of a slightly different nature from those encountered earlier in stabilisation of RUTF. This is because the proportion of fat is higher and the micronutrients are much more concentrated in the iLiNS supplements, with 10–40 g daily doses, compared with a daily dose of RUTF (~180–280 g).

Ongoing testing and quality control are critical to ensure that LNS products remain stable over time and under storage conditions encountered in the field. To evaluate the shelf life of iLiNS supplements, ongoing stability studies are run. These monitor physical stability (absence of oil separation), chemical stability (stability of the nutrients in the matrix over time) as well as sensory and organoleptic characteristics. Currently, the iLiNS Project supplements have a shelf life of 18 months, when stored at or below 30°C.

Current knowledge about the role of milk in LNS

Plumpy'Nut®, the first lipid-based RUTF, includes milk powder; this reflected the evolution of RUTF from earlier therapeutic milks used to treat SAM. Subsequently, as a variety of other products were developed (medium-quantity LNS/RUSF and the small-quantity products for prevention of growth faltering), some included milk powder while others did not.

Inclusion of milk products (generally DSM powder, but sometimes whey protein concentrate or sweet whey) also reflects the hypothesis that milk has a positive impact on linear growth, which is supported by some evidence (Mølgaard *et al*. 2011). Milk provides high-quality protein and several nutrients that are essential for growth, in highly bioavailable form. In addition, milk products may promote growth through other mechanisms, including stimulation of growth factors, as milk intake is associated with serum levels of insulin-like growth factor-1 (Hoppe *et al*. 2006; Dror & Allen 2011; Mølgaard *et al*. 2011). However, the minimum amount of milk required to stimulate growth is unknown (Michaelsen *et al*. 2009). Consequently, it is unknown whether (and how much) milk would be needed in a supplement that aims to prevent stunting through home fortification. As noted, the iLiNS studies and the iLiNS supplements evolved from previous research; the supplements in the previous studies included small amounts of DSM and/or whey. DSM and whey comprised 25–30% of the supplement weight, or 6–12 g/daily dose.

The quantity of milk in these early 'small-quantity' LNS was not a formulation goal, even though the growth-promoting effect of milk has been known for some time (Hoppe *et al*. 2006). It was not a goal partly because milk is the most expensive ingredient in milk-containing LNS. For this reason, in an attempt to reduce product cost, the iLiNS Project aims to examine the role of milk in these supplements. In two of three iLiNS sites (Ghana and Burkina Faso), the current studies provide milk-containing LNS to all study participants; but in one study in Malawi, iLiNS researchers are directly comparing LNS with and without milk. This study seeks to achieve our second project objective ('Evaluate the efficacy of reduced cost formulations of LNS'). The lowest LNS dose tested in Malawi (10 g day^{-1}) provides 2.4 g milk (in the form of DSM); the 20 g LNS provides 4.8 g and the 40 g LNS provides 9.6 g. The Malawi study will determine if this amount of milk provides any advantages for linear growth over isocaloric non-milk-containing LNS.

Selection of legumes and use of grains in LNS and implications for total energy dose

Most LNS include legumes among the ingredients; the legume in the original RUTF formulation was peanut. Currently, producers are formulating versions with a variety of legumes to suit local tastes where peanut is not common in the diet or is not locally available for LNS production. While a variety of legumes can be used, the fat and EFA content of legumes varies widely (Table 1). This variation in fat content will affect the contribution of legumes to total lipids and EFA in the LNS.

Some formulations have also included cereal grains as a major ingredient (see, e.g. Bisimwa *et al*. 2012). Like some legumes, cereal grains have very low fat content. Inclusion of substantial quantities of low-fat/low-EFA legumes and/or cereal grains means that the other ingredients (e.g. additional oil) must provide sufficient fat to meet EFA needs. This may require a higher daily energy ration of the product, with consequent increased possibility for displacement of breast milk, especially for younger infants.

As noted, iLiNS supplements were designed to enrich infant and maternal diets through targeted delivery of vitamins, minerals and EFA; that is, they were designed as 'home fortificants' to fill gaps between the home diet and nutrient needs. They were not designed to replace or displace local foods. Similarly, they were not designed for and are not adequate to meet energy needs in situations where sufficient staple foods are unavailable or inaccessible. In such food-insecure contexts, LNS could be provided along with cheaper unfortified food aid commodities to improve the nutrient density of staples and to render these adequate to meet the needs of targeted high risk groups (infants, pregnant and lactating women) (Chaparro & Dewey 2010). Thus, iLiNS supplements do not include lower fat legumes or grains as ingredients. Rather, caregivers are advised to mix the LNS into infant porridges typically made with locally available grains (and sometimes tubers). Pregnant and lactating women are advised to mix the LNS with any food of their choice.

In addition to varying content of fat (and type of fat), legumes and cereal grains also vary in content of certain anti-nutrients (see anti-nutrients, below).

Table 1. Total fat content of selected legumes*

Type of legume	Total fat g/ 100 g dry weight	Linoleic acid (n-6)	α-Linolenic acid (n-3)
Kidney bean, raw	0.83	0.18	0.28
Lentils, raw	1.06	0.40	0.11
Chickpea, raw	6.04	2.59	0.10
Soy bean, raw	19.94	9.93	1.33
Peanut, all types, raw	49.24	15.56	0.00

*Fat content of raw legumes from the USDA National Nutrient Database for Standard Reference (SR 23) accessed 18 September 2012 at: http://www.nal.usda.gov/fnic/foodcomp/search/. Values available for linoleic acid and α-linolenic acid are undifferentiated 18-2 and 18-3 fatty acids, respectively.

Sugar content

Sugar is added to infant LNS primarily to increase palatability. Some concerns have been raised about fostering infant preferences for sweet foods. Infants are born with an innate preference for sweet tastes, but need to learn to accept and develop preferences for a wide range of flavours early in life (Devaney & Fox 2008). Thus, it is sensible to try to minimise the sugar added to LNS while still ensuring that products are acceptable and palatable.

In the iLiNS supplements, the added sugar content of the 10 g LNS is very low (0.2 g) due in part to the need to allow 'space' for energy from EFA, as well as to fortify with target amounts of vitamins and minerals, within the 10 g. Other iLiNS supplements include slightly more added sugar; the 20 g supplements for infants include 1.6 g sugar, and the 40 g versions include 3.2 g sugar. The added sugar in the 20 g iLiNS supplements provides 1.1–3.1% of estimated energy needs from complementary foods (depending on age). Products with this sugar content were tested for acceptability in our three study sites and, as noted above, were well accepted by the infants in all sites (Adu-Afarwuah *et al*. 2011; Hess *et al*. 2011; Phuka *et al*. 2011).

Adult women in many cultures may prefer products that are less sweet than the ones preferred by infants and children. The iLiNS Project, as well as others, has experimented with different flavours for

LNS including more or less sugar. The resulting product for pregnant and lactating women in iLiNS contains slightly less sugar than the infant/young child products (1.2 g sugar as compared to 1.6 g, in a 20 g dose).

Anti-nutrients

LNS are designed to provide specified amounts of a range of micronutrients. However, anti-nutrients in LNS ingredients and in the local diet can affect the bioavailability of certain micronutrients. The most important of these anti-nutrients is phytate (phytic acid). Several other anti-nutrients may also be important, but are less widely distributed in foods (Michaelsen *et al*. 2009; Roos *et al*. 2013). Phytate levels are high in unrefined staple grains and legumes, which often dominate the diets of the poor, and phytate is a potent inhibitor of absorption of minerals including iron, zinc and calcium (Michaelsen *et al*. 2009).

Phytate content therefore needs to be considered when determining the quantity of added minerals in LNS, so that the supplement provides the desired levels of absorbable iron, zinc and calcium. Molar ratios of phytate to minerals are used to assess potential inhibitory effects of phytate on absorption. Molar ratios in all iLiNS supplements are acceptably low (e.g. phytate : zinc and phytate : iron molar ratios are all <1; phytate : calcium ratios are all ≤0.01) (Gibson *et al*. 2010).

However, in addition to considering the phytate content of the LNS ingredients themselves, those formulating LNS intended to be mixed with infant porridges should also consider the level of phytate in typical preparations of local infant porridges. Similarly, the overall phytate content in women's diet needs to be considered in setting target levels for minerals in LNS for pregnant and lactating women. This is difficult to fully predict due to the wide variety of foods/combinations with which the LNS may be consumed. In the iLiNS Project, mineral levels were set taking into account likely diet patterns; and iron and zinc status will be assessed in study subjects.

Tannins are also present in large quantities in some grains and legumes, with content varying widely. Tannin content is signalled by the colour of the unrefined grain or legume, with dark-coloured grains (e.g. sorghum and millet) and dark-coloured legumes (e.g. fava beans) having high content and light-coloured legumes (e.g. soy) having very low levels of tannins. Like phytate, tannins interfere with absorption of minerals, most importantly of iron and zinc (Michaelsen *et al*. 2009), and as with phytate, levels of tannins and other anti-nutrients in ingredients should be considered during formulation of LNS.

Box 4. Key considerations in LNS design – energy dose

- iLiNS supplements are small-quantity (20 g) LNS[a] designed to enrich diets ('home fortification').
- Energy dose of LNS should be selected considering amount of energy infants need from foods other than breast milk, with the aim of avoiding displacement of breast milk.
- LNS should be designed to avoid displacing a diverse diet of available/accessible local nutrient-dense foods.

[a]Exception: in one of four iLiNS trials, a larger (40 g) dose is compared to 10- and 20-g doses.

Considerations for selecting energy dose per day

Infants and young children under 2 years

As noted, small-quantity LNS are intended to enrich infant diets through 'home fortification' of local foods (Box 4). Even with a diverse diet, fortified products may be necessary because of limited economic access to sufficient quantities of nutrient-dense foods, especially animal-source foods (Pan American Health Organization/World Health Organization 2003). For infants 6–11 months of age, who consume small quantities of complementary food, desired nutrient density is particularly high and gaps between intakes and needs are difficult to fill without fortified products (Dewey & Brown 2003; Vitta & Dewey 2012). Similar to MNP for home fortification, small quantities of LNS can be mixed with porridges or other complementary food. Unlike MNP, LNS may also be eaten as is (directly from the packet). In the iLiNS studies, mothers are advised to mix the LNS with porridge or other food. However, early experience in our studies

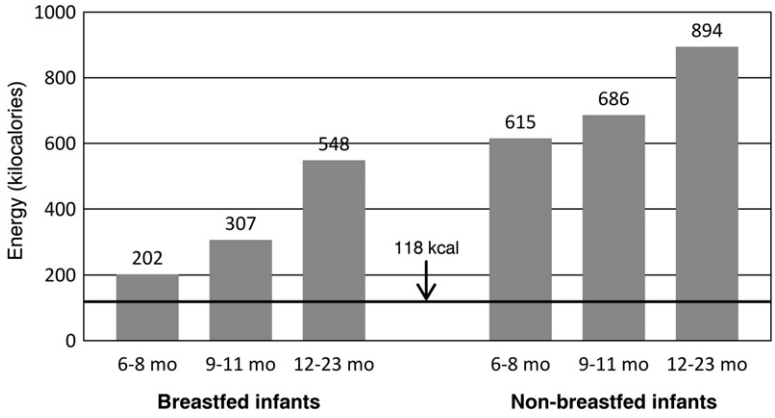

Fig. 1. Energy in iLiNS Project 20-g supplement compared to energy needs from complementary foods (breastfed infants) or total energy needs (non-breastfed infants). Estimated energy needs and breast milk intakes are from Dewey & Brown (2003). For breastfed infants, average breast milk intake is assumed.

shows that, nevertheless, LNS is sometimes given as is, especially as infants get older. This has also been observed in other studies (Tripp *et al*. 2011).

Ideally, infants are breastfed for 2 years or longer, with complementary food introduced at 6 months. From 6 to 11 months, diets of infants and young children should gradually become more diverse, to include a variety of available fruits, vegetables and animal-source foods. All infants need to learn to eat and enjoy locally available nutrient-dense foods, especially because diet patterns and preferences formed early in life may persist (Devaney & Fox 2008; Nicklaus 2009; Beauchamp & Mennella 2011). Neither breast milk nor a diverse diet of local foods should be displaced by LNS or any other fortified product.

To date, two studies have examined breast milk intake of infants given LNS (25 or 50 g day^{-1}, providing 128–275 kcal) compared with infants given a fortified-blended complementary food (FBF), and found no displacement of breast milk relative to the FBF (Galpin *et al*. 2007; Owino *et al*. 2011). Average breast milk intakes in both studies were higher than global averages for developing countries (World Health Organization 1998). Four other studies did not assess breast milk intake but reported that frequency of breastfeeding was not decreased for infants given LNS (Adu-Afarwuah *et al*. 2007b; Flax *et al*. 2008, 2010); breastfeeding frequency increased in one study (Paul *et al*. 2012). Nevertheless, because breastfeeding is critical to child survival and continues to provide nutrient-dense, high-quality nourishment for infants to 2 years and beyond, additional studies across various age groups and settings are needed to confirm that LNS does not displace or decrease breast milk intake. The iLiNS study in Malawi is quantitatively assessing breast milk intake among infants at 9–12 months of age who have been given 0, 10, 20 or 40 g of LNS (daily dose) beginning at 6 months of age.

Assuming average breast milk intake, complementary foods should provide ~200 kcal at 6–8 months, increasing to ~300 at 9–11 months and ~550 kcal at 12–23 months. To our knowledge, no study to date has quantitatively examined the impact of consumption of LNS on intake of local complementary foods. iLiNS Project studies in Malawi and Ghana are assessing this, with assessment of food group consumption and diversity in all sites.

To avoid displacement of breast milk and of available local foods, iLiNS 20 g supplements provide 118 kcal day^{-1}, or approximately one-half of the complementary food energy requirements of healthy infants 6–8 months of age, approximately one-third for infants 9–11 months, and one-fifth of the complementary food energy required at 12–23 months (Fig. 1). In one study site (Malawi), iLiNS researchers are comparing 10-, 20- and 40-g supplements with the objective of identifying the lowest dose and lowest cost supplement that is still efficacious. The rationale for the 40-g supplement in this setting is that in one of the previous studies, a larger (50 g) supplement reduced severe stunting, and this effect was sustained over several years (Phuka *et al*. 2008, 2009b).

Pregnant and lactating women

As with infants, iLiNS supplements for adult women are designed to enrich diets with micronutrients and EFA. Small-quantity LNS products are not designed to fill energy gaps; where these gaps exist, they can be filled by local foods at much lower cost. In emergency settings where local foods are unavailable, energy gaps could still be filled by unfortified commodities at much lower cost than by LNS or other specialised products.

In contrast to the situation for infants, consumption of LNS by pregnant and lactating women generally does not raise concerns about displacement of other nutrient-dense foods, as energy from small-quantity LNS would provide a very small proportion of requirements. Therefore the daily energy dose should be chosen based on providing the minimum food base required to deliver EFA and the need to mask strong-tasting macrominerals (e.g. magnesium and potassium). In the iLiNS formulation for pregnant and lactating women, this was achieved with a 20-g daily dose.

LNS and the nutrition transition

As noted, small-quantity LNS used to home-fortify local diets are not designed to fill energy gaps in food-insecure settings. They may be used to fill gaps in intakes of micronutrients and EFA, including in contexts where excess energy intakes and resulting overweight and obesity are public health concerns. Because LNS are high in fat and energy-dense, this raises the issue of the impact of adding an energy-dense food to local diets.

The issue is different for infants than for pregnant and lactating women. There is no evidence that the level of dietary fat consumed during infancy contributes to overweight and obesity (Macé *et al*. 2006; Rolland-Cachera *et al*. 2012), although there may be concern around developing preferences for energy-dense foods (Devaney & Fox 2008; Nicklaus 2009; Beauchamp & Mennella 2011). Breast milk derives ~50% of its energy from fat, and recommendations for infants are that fat intake should gradually decrease from 40% to 60% during exclusive breast-feeding to 35% at 2 years of age (Food and Agriculture Organization 2010). Consumption of 20 g of LNS (iLiNS formulation) provides 9.6 g of fat. This allows for other fat in the diet of infants, but could contribute to excessive intakes if fat intake from other family foods and/or fried snacks was excessively high.

For adult women, the potential contribution of an additional energy-dense food to overweight or obesity may be a concern in the context of the nutrition transition. Whether or not consumption of LNS during pregnancy and lactation – when energy needs are elevated – would contribute to unhealthy weight gain or retention is unknown. The only previously published study on use of LNS during pregnancy (Huybregts *et al*. 2009) was in an area where maternal underweight, but not overweight, was a significant concern. In the current iLiNS trials, weight gain during pregnancy and post-partum weight retention will be assessed including in one site (Ghana) where overweight and obesity are a very significant public health concern.

For both infants and women, these issues, as well as cost issues, provide a rationale for minimising the food base used to deliver micronutrients, EFA and high-quality protein. The iLiNS infant supplements being evaluated include a very small-quantity supplement (10 g, providing 4.8 g fat) as well as the more typical 20- and 40-g daily quantities, and the iLiNS women's supplements were formulated using the minimum necessary food base, given technical considerations and taste.

Considerations for selecting micronutrient and essential fatty acid content

The nutrient composition of all iLiNS products is based on the assumption that in resource-poor settings, diets of vulnerable infants, young children, and pregnant and lactating women are likely to be deficient in multiple micronutrients (Gibson & Hotz 2000; Dewey & Brown 2003; Arimond *et al*. 2010; Chaparro & Dewey 2010; Torheim *et al*. 2010; Lee *et al*. 2012) (Box 5). Except when consumption of fish and/or high-EFA oils is common, diets of the poor are also likely to be deficient in EFA (Michaelsen *et al*.

Box 5. iLiNS supplements: key considerations for nutrient content

- iLiNS supplements are designed for settings where vulnerable groups are likely to have multiple micronutrient deficiencies.
- iLiNS supplements for infants provide approximately 1 RDA or AI[a] of many micronutrients, as well as of essential fatty acids.
- In addition to 'Type I' nutrients, iLiNS supplements also provide 'Type II' nutrients including several macrominerals and zinc.[b]
- Iron levels and recommendations for iLiNS supplement use were designed to minimise any risk of iron consumption, particularly in the malaria-endemic iLiNS study areas; in addition, we monitor and ensure care for malaria and other infections.
- Micronutrient levels in iLiNS supplements for women were influenced by recent supplementation trials of multiple micronutrients during pregnancy.

[a]RDA, Recommended Dietary Allowances; AI, Adequate Intakes.

[b]Type I nutrients (vitamins, calcium, iron, iodine, selenium) are needed for specific biochemical functions; a deficiency results in clinical signs/symptoms but not necessarily in reduced growth. Type II nutrients (protein, sulphur-containing amino acids, magnesium, phosphorus, potassium, sodium, zinc) are the building blocks of tissue. If there is a deficiency in any of them, the child will exhibit reduced growth (Golden 1995).

2011). In these same settings, micronutrient needs may be elevated due to pre-existing deficiencies as well as acute and/or chronic infections, which cause increased nutrient losses as well as sequestration of nutrients (Golden 2009; Dewey & Mayers 2011). Typical diet patterns, dominated by staple foods and low in animal-source foods, result in low bioavailability for some micronutrients (e.g. iron, zinc and provitamin A carotenoids).

Tables 2 and 3 show the micronutrient and EFA content of the LNS formulated for use in the iLiNS Project for infants/young children (20-g dose) and women, respectively. For infants/young children, the 10-, 20- and 40-g doses differ only in macronutrient content.

Tables 2 and 3 also show Institute of Medicine (IOM) Dietary Reference Intakes (DRI) and World Health Organization/Food and Agriculture Organization (WHO/FAO) Recommended Nutrient Intakes (RNI) as points of reference. Note that these sometimes differ and the DRI may assume (as for iron and zinc) diet patterns that are not typical in poor households in developing countries (World Health Organization/Food and Agriculture Organization 2004; Otten *et al*. 2006). Table 4 indicates the chemical forms of each micronutrient recommended for fortified complementary foods and supplements, and used in iLiNS products.

Infants and young children under 2 years

The nutrient composition of iLiNS supplements for infants and young children was based on Nutributter®, the product used in the previous trial in Ghana (Adu-Afarwuah *et al*. 2007b). Nutributter® was designed so that the daily dose would generally provide the WHO/FAO RNI for the key micronutrients for infants 7–12 months of age because this was the age range in the Ghana study. Because of technical difficulties associated with adding the desired amounts of the four macrominerals (calcium, potassium, magnesium and phosphorus), the amounts included in Nutributter® were lower than the WHO/FAO RNI. The magnesium, manganese and potassium contents were contributed by the food ingredients only. Iron content in Nutributter® was based on an assumed bioavailability of 10%, and zinc content was based on an assumption of moderate bioavailability.

For the iLiNS supplements, several additional nutrients (not added to Nutributter®) were added as fortificants: vitamins K, D, E, and potassium, magnesium and manganese. The levels of copper and selenium were increased to match the IOM DRI value for 12–23 months because the iLiNS intervention periods extend to 18 months of age. This was not done for the B vitamins because it was assumed that the additional requirement for 12–23 months (above the RNI for 7–12 months) would be contributed by the B vitamins in breast milk and other complementary foods. The levels of calcium, potassium, magnesium and phosphorus were increased to the extent possible given technical limitations.

The level of zinc was increased to 8 mg (the 'low' bioavailability value in the WHO/FAO RNI) because of the observation that plasma zinc levels in the earlier Ghana trial did not respond to the 4 mg of zinc

Table 2. Nutributter and iLiNS formulations for infants/young children, in comparison to US Institute of Medicine (IOM) Adequate Intakes (AI) or Recommended Dietary Allowances (RDA) and World Health Organization/Food and Agriculture Organization(WHO/FAO) Recommended Nutrient Intakes (RNI)[†]

Nutrient	Unit	US Dietary Reference Intakes			WHO/FAO RNI		Nutributter®	iLiNS 20 g[‡]	Comment
		AI/RDA 7–11 months	AI/RDA12–23 months	UL	7–12 months	1–3 years			
Dose	g						20	20	
Energy	kcal						108	118	
Protein	g	11	13	–	10.5	11.9	2.56	2.6	WHO/FAO requirements are stated separately for boys and girls, and at 0.5, 1, 1.5, 2 and 3 years. The values shown are averages across age and sex.
Fat	g	30*	30–40% E[§]	–	See comment		7.08	9.6	Food and Agriculture Organization (2010) reduce gradually from 40–60% (up to 6 months) to 35% at 24 months and an AMDR of 25–35% for 2 years and older.
Linoleic acid	g	4.6*	7*	–	3.0–4.5% E		1.29	4.46	The FAO AI is for 6–24 months.
α-Linolenic acid	g	0.5*	0.7*	–	0.4–0.6% E		0.29	0.58	
Vitamin A	μg	500*	300	600	400	400	400	400	Unit for US RDA is retinol activity equivalents (RAE) and UL is for retinol only, not carotenoids; WHO/FAO RNI unit is RE; for iLiNS these are equivalent as the fortificant is retinol.
Thiamin (B1)	mg	0.3*	0.5	–	0.3	0.5	0.3	0.3	WHO/FAO UL of 10.
Riboflavin (B2)	mg	0.4*	0.5	–	0.4	0.5	0.4	0.4	WHO/FAO UL of 30.
Niacin (B3)	mg	4*	6	–/10	4	6	4	4	
Pantothenic acid (B5)	mg	1.8*	2*	–	1.8	2	1.8	1.8	
Vitamin B6	mg	0.3*	0.5	–/30	0.3	0.5	0.3	0.3	
Vitamin B12	μg	0.5*	0.9	–	0.7	0.9	0.5	0.5	
Folic acid	μg	80*	150	–/300	80	150	80	80	Requirement is in DFE; 1 DFE = 1 μg food folate; 1 DFE = 0.6 μg folic acid from fortified foods.
Vitamin C	mg	50*	15	–/400	30	30	30	30	
Vitamin D	μg	10*	15	37/62	5	5	0	5	US AI was 5 μg when iLiNS supplements were formulated in 2009; current WHO/FAO requirement (2004) is also 5 μg.
Vitamin E	mg	5*	6	–/200	2.7	5	0	6	Target was RDA at 12–23 months.
Vitamin K	μg	2.5*	30	–	10	15	0	30	Target was RDA at 12–23 months.
Calcium	mg	260*	700	1500/2500	400	500	100	280	US AI was 270 for 6–11 months and 500 for 1–3 years in 2009 when iLiNS supplements were formulated in 2009.

Table 2. Continued

Nutrient	Unit	US Dietary Reference Intakes			WHO/FAO RNI		Nutributter®	iLiNS 20 g‡	Comment
		AI/RDA 7–11 months	AI/RDA12–23 months	UL	7–12 months	1–3 years			
Copper	mg	0.22*	0.34	–/1.0	–	–	0.2	0.34	No WHO/FAO RNI.
Iodine	μg	130*	90	–/200	90	90	90	90	WHO/FAO UL of 140 μg kg^{-1} day^{-1} for 7–11 months and 50 μg $kcal^{-1}$ day^{-1} for 1–3 years.
Iron	mg	11	7	40	6.2	3.9	9	6	US RDA assume absorption as from US mixed diet. WHO/FAO RNI are shown for varying assumptions about bioavailability (15% at top, 12%; 10%; and 5%). iLiNS set below RDA and below Nutributter to reduce possible risk in malaria-endemic areas.
					7.7	4.8			
					9.3	5.8			
					18.6	11.6			
Magnesium	mg	75*	80	–/65	54	60	16	40	UL is for pharmacological agents only, not food/water; RNI could not be attained for technical reasons.
Manganese	mg	0.6*	1.2*	–/2.0			0.08	1.2	No WHO/FAO RNI.
Phosphorus	mg	275*	460	–/3000			82.2	190	No WHO/FAO RNI; RDA could not be attained for technical reasons.
Potassium	mg	700*	3000*	–			152	200	No WHO/FAO RNI; RDA could not be attained for technical reasons.
Selenium	μg	20*	20	60/90	10	17	10	20	
Zinc	mg	2.5	3	5/7	2.5	2.4	4	8	US RDA assumes absorption as from US mixed diet; WHO/FAO RNI are shown for diets with high, moderate and low bioavailability. The WHO/FAO UL for both age groups is 23–28.
					4.1	4.1			
					8.4	8.3			

AI, Adequate Intakes and are denoted with an '*'; RDA, Recommended Dietary Allowances; UL, Tolerable Upper Intake Levels; '–', indicates not determinable or data insufficient; where two values are given, first is for 6–11 months, second for 12–23 months. When different, WHO/FAO UL is given in the comment column. †US Dietary Reference Intakes accessed at http://www.iom.edu/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx (28 September 2012). Historical vitamin D dietary reference intakes are from Otten *et al.* (2006). WHO/FAO requirements are from World Health Organization/Food and Agriculture Organization (2004). Nutributter® nutrient content is provided by the producer (Nutriset LLC). ‡The micronutrient content of the 10- and 40-g iLiNS LNS for infants/young children is identical to the 20-g formulation. However, as the larger dose supplements contain more food base, the balance between micronutrients intrinsic to the food ingredients vs. micronutrients added differs for the three formulations. The concentration of added vitamins/minerals is approximately eight times higher in the 10-g than in the 40-g supplement. The fat, EFA, and protein content are proportional by weight and therefore differ between the 10-, 20- and 40-g supplements. For example, the iLiNS 10-g supplement has 4.8 g of total fat, and the 40-g dose has 19.2 g fat. §30 g is AI for 6–11 months; 30–40% of energy is acceptable macronutrient distribution range for 12–23 months.

Table 3. iLiNS formulation for pregnant and lactating women, in comparison to US Institute of Medicine (IOM) Adequate Intakes (AI) or Recommended Dietary Allowances (RDA), World Health Organization/Food and Agriculture Organization (WHO/FAO) Recommended Nutrient Intakes (RNI), and UN international multiple micronutrient preparation (UNIMMAP)†

Nutrient	Unit	US Dietary Reference Intakes			WHO/FAO RNI		UNIMMAP	iLiNS P & L	Comment
		AI/RDA Pregnancy (19–50 years)	AI/RDA Lactation (19–50 years)	UL	Pregnancy (3rd trimester)	Lactation 0–3/0–6 months if different			
Dose	g						0	20	
Energy	kcal						0	118	
Protein	g						0	2.6	US RDA: 1.1 g kg^{-1} day^{-1} for second half of pregnancy; 1.3 lactation. Else 0.80 for adult women.
Fat	g	20–35% E	20–35% E	–	20–35% E	20–35% E	0	10	
Linoleic acid	g	13*	13*	–	–	–	0	4.59	
α-Linolenic acid	g	1.4*	1.3*	–	–	–	0	0.59	
Vitamin A	μg	770	1300	3000	800	850	800	800	Unit for US RDA is retinol activity equivalents (RAE) and UL is for retinol only, not carotenoids; WHO/FAO RNI unit is RE; for iLiNS these are equivalent as the fortificant is retinol.
Thiamin (B1)	mg	1.4	1.4	–	1.4	1.5	1.4	2.8	
Riboflavin (B2)	mg	1.4	1.6	–	1.4	1.6	1.4	2.8	
Niacin (B3)	mg	18	17	35	18	17	18	36	
Pantothenic acid (B5)	mg	6*	7*	–	6	7	0	7	
Vitamin B6	mg	1.9	2.0	100	1.9	2.0	1.9	3.8	
Vitamin B12	μg	2.6	2.8	–	2.6	2.8	2.6	5.2	
Folic acid	μg	600	500	1000	600	500	400	400	Requirement is in DFE; 1 DFE = 1 μg food folate; 1 DFE = 0.6 μg folic acid from fortified foods.
Vitamin C	mg	85	120	2000	55	70	70	100	
Vitamin D	μg	15	15	100	5	5	5	10	IOM AI was also 5 μg when we formulated iLiNS supplements in 2009.
Vitamin E	mg	15	19	1000	–	–	10	20	No WHO/FAO RNI.
Vitamin K	μg	90*	90*	–	55	55	0	45	Limited due to cost.
Calcium	mg	1000*	1000*	2500	1200	1000	0	280	Maximum possible within technical constraints.

Table 3. Continued

Nutrient	Unit	US Dietary Reference Intakes			WHO/FAO RNI		UNIMMAP	iLiNS P & L	Comment
		AI/RDA Pregnancy (19–50 years)	AI/RDA Lactation (19–50 years)	UL	Pregnancy (3rd trimester)	Lactation 0–3/0–6 months if different			
Copper	mg	1.0	1.3	10	–	–	2.0	4	No WHO/FAO RNI.
Iodine	μg	220	290	1100	–	200	150	250	Based on WHO recommendations at the time the iLiNS supplement was developed.
Iron	mg	27	9	45	–	10	30	20	US RDA assume absorption as from US mixed diet; no WHO/FAO RNI during pregnancy; RNI for lactation are shown for varying assumptions about bioavailability (15% at top, 12%; 10% and 5%).
						12.5			
						15			
						30			
Magnesium	mg	350/360‡	310/320‡	350	220	270	0	65	Maximum possible within technical constraints; UL is for pharmacological agents only, not food/water.
Manganese	mg	2.0*	2.6*	11	–		0	2.6	No WHO/FAO RNI.
Phosphorus	mg	700	700	3500/4000	–	–	0	190	Maximum possible within technical constraints; no WHO/FAO RNI.
Potassium	mg	4700*	5100*	–	–	–	0	200	Maximum possible within technical constraints; no WHO/FAO RNI.
Selenium	μg	60	70	400	30	35	65	130	
Zinc	mg	11	12	40	6	5.8/5.3	15	30	US RDA assume absorption as from US mixed diet; the WHO/FAO RNI are shown for diets with high, moderate, and low bioavailability.
					10	9.5/8.8			
					20	19/17.5			

AI, Adequate Intakes and are denoted with an '*'; RDA, Recommended Dietary Allowances; UL, Tolerable Upper Intake Levels; UNIMMAP, UN international multiple micronutrient preparation; '–', indicates not determinable or data insufficient; where two values are given, first is for pregnancy and second is for lactation. †US Dietary Reference Intakes accessed at http://www.iom.edu/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx (28 September 2012). Historical vitamin D and calcium dietary reference intakes are from Otten *et al.* (2006). WHO/FAO requirements are from World Health Organization/Food and Agriculture Organization (2004). UNIMMAP supplement nutrient content is from Margetts *et al.* (2009). ‡Values for ages 19–30 years/31–50 years.

Table 4. Chemical forms of nutrients in iLiNS products and recommended forms

Micronutrient	Used in iLiNS formulations	Recommended chemical forms*
Vitamin A	Retinyl acetate	Retinyl acetate or retinyl palmitate or β-carotene
Thiamin (B1)	Thiamin hydrochloride	Thiamin mononitrate (preferred for dry products) or thiamin hydrochloride
Riboflavin (B2)	Riboflavin	Riboflavin
Niacin (B3)	Niacinamide	Niacinamide
Pantothenic acid (B5)	Calcium pantothenate	
Vitamin B6	Pyridoxine hydrochloride	Pyridoxine hydrochloride
Vitamin B12	Cyanocobalamin (0.1%)	Cyanocobalamin (diluted form (0.1% or 1%) with 100% active particles, spray dried form)
Folic acid	Pteroyl monoglutamic acid	Pteroyl monoglutamic acid
Vitamin C	L-ascorbic acid	L-ascorbic acid
Vitamin D	Cholecalciferol (D3)	Ergocalciferol (D2) or cholecalciferol (D3)
Vitamin E	DL-alpha-tocopherol acetate	Acetates of D or DL-alpha-tocopherol
Vitamin K1	Phylloquinone 5%	
Calcium	Tricalcium phosphate	Several forms available; some with higher contents of calcium, such as calcium phosphate and calcium carbonate; soluble organic calcium salts such as calcium citrate. Calcium salts containing well absorbed anions (such as chloride) should be avoided as they may induce acidosis
Copper	Encapsulated copper sulphate	Copper sulphate or copper gluconate
Iodine	Potassium iodate	Potassium iodate
Iron	Encapsulated ferrous sulphate	NaFeEDTA (subject to Codex limits), encapsulated ferrous sulphate, encapsulated ferrous fumarate and micronised ferric pyrophosphate could also be used but costs need to be considered
Magnesium	Magnesium citrate	Soluble organic magnesium salts such as Mg citrate. Magnesium salts containing well absorbed anions (such as chloride) should be avoided as they may induce acidosis
Manganese	Manganese sulphate	
Phosphorus	Tricalcium phosphate Dipotassium phosphate	
Potassium	Potassium chloride Dipotassium phosphate	
Selenium	Sodium selenite 1.5%	Sodium selenate or sodium selenite
Zinc	Zinc sulphate	Zinc sulphate, zinc gluconate, zinc oxide

*The third column in this table lists recommendations from the Ten Year Strategy to Reduce Vitamin and Mineral Deficiencies, Maternal, Infant and Young Child Nutrition Working Group: Formulation Subgroup (2009). The primary source for the recommendations was Allen *et al*. (2006).

included in Nutributter® (Adu-Afarwuah *et al*. 2008).

The level of iron was decreased from 9 mg (in Nutributter®) to 6 mg (in iLiNS supplements) because of concern that higher amounts of iron might cause adverse effects, especially in malaria-endemic areas for which specific guidance was provided by WHO (World Health Organization 2007). In addition, caregivers in the iLiNS trials are advised to divide the daily dose of LNS into two meals so that the amount of iron per meal would be ~3 mg, a level comparable to the amount per meal provided by fortified processed complementary foods. In the statement emerging from the WHO technical consultation in 2006, processed complementary foods fortified with iron were considered to be safe because they provide a physiological dose of iron distributed throughout the day, 'which avoids the adverse gastrointestinal and morbidity effects of a bolus dose' (World Health Organization 2007, p. S625).

Pregnant and lactating women

The micronutrient composition of the LNS developed for pregnant and lactating women in the iLiNS Project is similar to that of other multiple micronutrient prenatal supplement formulations, such as that developed by UNICEF, WHO and the United

Nations University [the U.N. international multiple micronutrient preparation (UNIMMAP) (Margetts *et al*. 2009)], with some modifications. Levels of certain nutrients (thiamin, riboflavin, niacin, vitamin B_6, vitamin B_{12}, vitamin D, vitamin E, zinc, copper and selenium) were increased to twice the amount in the UNIMMAP formulation because this resulted in better pregnancy outcomes in Guinea-Bissau (Kaestel *et al*. 2005). Moreover, providing the Recom-Mended Dietary Allowances (RDA) to pregnant women in rural Nepal failed to increase the maternal serum concentrations of micronutrients to normal values (Christian *et al*. 2006). However, several nutrients were not doubled in our LNS. Vitamin A was not doubled due to concerns with risk of transmission of HIV (Fawzi *et al*. 1998; Humphrey *et al*. 2006); vitamin C was increased but could not be doubled due to technical constraints (palatability); folic acid was not doubled to avoid any additional risk of interfering with anti-folate drugs used in the treatment of malaria; and iodine was not doubled but was increased (relative to UNIMMAP) to be consistent with a more recent WHO recommendation. Other modifications included addition of several nutrients (pantothenic acid, vitamin K, calcium, phosphorus, potassium, magnesium, iodine and manganese) that were not included in the original UNIMMAP formulation or in Guinea-Bussau because of cost or feasibility.

Finally, the iron content of the LNS for pregnant and lactating women was set at 20 mg, which is lower than the 30 mg in the UNIMMAP formulation designed for pregnant women (and also lower than the 30 mg used in the Guinea-Bissau trial). For practical purposes, it may be preferable to have just one LNS product for both pregnant and lactating women. For most nutrients, the recommended intakes for these two target groups are similar. However, for iron, the IOM RDA is 27 mg for pregnant women and 9 mg day^{-1} for lactating women. Because we did not want to greatly exceed the RDA during lactation, we chose a target value of 20 mg of iron for both groups. Studies have indicated that 20 mg day^{-1} is an adequate dose to prevent iron deficiency anaemia during pregnancy (even for women who are iron deficient at entry to prenatal care) and causes fewer gastrointestinal side effects compared with higher doses of iron (Milman *et al*. 2006; Zhou *et al*. 2009). Studies of iron absorption using stable isotopes have shown that absorption is 24–66% during the third trimester of pregnancy, when modest doses of iron (6–18 mg) are given with food daily (Barrett *et al*. 1994; Whittaker *et al*. 2001; Woodhouse *et al*. 2008). The requirement for *absorbed* iron is estimated to be ~4–5 mg day^{-1} in the second trimester and ~5–6 mg day^{-1} in the third trimester (Institute of Medicine 2001). We assumed that if the women in our study areas obtained ~18 mg day^{-1} from their normal diet (Ndekha 1998) and per cent absorption was at least 10%, they would be getting at least 2 mg day^{-1} of absorbed iron from the home diet and would thus need another 2–4 mg day^{-1} from LNS. LNS with 20 mg of iron, assuming at least 25% absorption, would deliver at least 5 mg of absorbed iron and should thus be sufficient for iron needs in late pregnancy, when demand is highest.

Overlap with fortification and supplementation programmes

In formulating the iLiNS supplements, or any supplement meant to be consumed on a daily basis, the possibility of excess intakes must be considered. This can be considered first in relation to the nutrient content of the supplement and second in relation to projected intakes from all sources, including other fortified products and supplements.

The IOM and WHO/FAO provide guidance in the form of Tolerable Upper Intake Levels (UL), which are defined as 'the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population and applies to daily use for a prolonged period of time' (Otten *et al*. 2006). UL, by design, incorporate safety factors such that each UL is generally set well below the level where actual adverse effects have been observed.

With few exceptions, the nutrient content of the iLiNS supplements is below, and usually well below, the UL (see Tables 2 and 3). The exceptions are:

1. The zinc content of the infant supplement exceeds the IOM UL but not the WHO/FAO UL. In this case, the WHO/FAO UL is more relevant because the IOM UL relates to a US diet, with much higher zinc bioavailability assumed; and
2. The niacin content of the maternal supplement. However, adverse effects are not associated with intake of the nicotinamide form of niacin, which is the chemical form included in the iLiNS supplements. In addition, the same niacin content was used in the Guinea-Bissau trial, with no apparent adverse effects.

Concerning the potential to exceed UL when total intake is considered, this risk has been considered carefully in relation to MNP that provide an RDA dose of up to 15 micronutrients. The Home Fortification Technical Advisory Group (2011) concluded that MNP can be safely provided in addition to twice-yearly high-dose vitamin A supplements, iodised salt and general food fortification. However, they advise that MNP should not be consumed at the same time as other specially formulated products (e.g. RUTF or LNS). Chaparro & Dewey (2010) also provided an extensive discussion of potential risks, on a nutrient-by-nutrient basis, and concluded that daily consumption of LNS similar to the iLiNS supplements is safe. There is no guidance available on use of antenatal LNS because the iLiNS efficacy trials are among the first to explore this approach. Women receiving antenatal LNS in the iLiNS trials generally do not receive iron-folic acid tablets from their health care providers because the antenatal LNS includes the same amount of folic acid as recommended by WHO (400 μg) and the amount of iron (20 mg) was calculated to be sufficient if the product is consumed daily.

Safety and quality

Applicable food safety and Codex Alimentarius standards

Production of LNS should meet high-quality standards and follow applicable guidance, regulations and protocols including implementation of the Hazard Analysis Critical Control Points (HACCP) method and the application of Good Manufacturing Practices. Recommendations of the following general International Organization for Standardization (ISO) and Codex Alimentarius standards are applied during production of all iLiNS supplements (Box 6):

Box 6. Key considerations for product safety and quality

- Raw ingredients and production processes for LNS must meet high-quality standards to ensure product safety.
- LNS raw ingredients and finished products can be contaminated with either toxins (e.g. aflatoxin) or microbes; contamination issues are not unique to LNS.
- Post-production testing is required to confirm and document product stability and nutrient content over time.
- Peanut allergy is a high-profile issue in the United States and some other developed countries, but prevalence of allergy is no higher than for milk, and the American Association of Pediatrics currently recommends no avoidance of peanut (or other potential allergens) after 6 months of age.
- iLiNS Project supplements meet all applicable quality and safety standards and are tested for contaminants, stability and nutrient content at multiple time points.

• ISO 22000:2005: Food safety management systems – Requirements for any organization in the food chain;
• Recommended International Code of Practice. General Principles of Food Hygiene (CAC/RCP 1-1969, Rev. 4-2003);
• Guidelines on formulated supplementary foods for older infants and young children (CAC/GL 08-1991);
• Report of the 28th session of the Codex committee on nutrition and foods for special dietary uses (CCNFSDU). Chiang Mai, Thailand, 30 October–3 November 2006 (Alinorm 07/30/26);
• Advisory List of Mineral Salts and Vitamin Compounds for Use in Foods for Infants and Children (CAC/GL 10-1979); and
• Codex Alimentarius standards existing for each individual raw material.

Revision of relevant Codex Alimentarius texts is currently underway in response to the development of new complementary foods and complementary food 'fortificants', including LNS products, in recent years. Following a review of all relevant Codex documents

addressing child feeding, it was suggested that the Codex guidelines on formulated supplementary foods for older infants and young children (CAC/GL 08-1991) be revised and updated in order to incorporate new products such as LNS. The delegation from Ghana initiated the revision process for these guidelines through the CCNFSDU in 2008. The guidelines have since gone through a series of revisions, and the latest revision of the document was adopted by CCNFSDU at step 8 (the final step in the Codex process) and will be forwarded to the Codex Commission for their adoption in mid-2013.

Potential hazards

Challenges related to contamination are not unique to production of LNS. Many food ingredients and finished products can potentially be contaminated before, during or after processing; thus, appropriate quality control is needed throughout.

The following describes procedures related to quality and safety applied during the production of iLiNS Project supplements. Consistent with safety management standards in ISO 22000, procedures related to raw ingredients are strictly followed by procurement, quality and production staff.

A validation file accompanies each ingredient. It consists of a technical data sheet from the supplier, a set of specific nutrient/chemical targets (set by the LNS producer), a statement on content of potentially allergenic material in the selected ingredient, a statement on the geographic origin of the ingredient, a document on specific hazards, a certificate of absence of contaminants (including, at a minimum, GMOs, dioxins, radioactivity, heavy metals and pesticides), a certificate on the type of sanitary agreement used (HACCP, ISO, Good Manufacturing Practices, etc.), a certificate of analyses performed on a sample of the ingredient (these may include analysis for aflatoxin, full microbiological analysis on milk ingredients, fatty acid profile and peroxide value on fats, etc., depending on the ingredient) and, finally, results of tests conducted on a representative sample of a recent production trial by the supplier. When the validation file is complete, the procurement department can place the order for the validated ingredient.

Then, upon receipt by the production unit, a specific control plan is followed for each ingredient. This may consist of sensory evaluation, and microbiological (salmonella), peroxide value or aflatoxin measurement depending on the type of ingredient. Every batch of finished product undergoes a range of physicochemical and microbiological analysis. These analyses are included on certificates of analyses (CoA) sent to partners/clients: proteins, fat, chlorides (as a tracer of homogeneity of the product), mesophil aerobic flora, yeasts, moulds, spores of sulphite-reducing bacteria, *Listeria*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and aflatoxins. Beginning in September of 2012, testing and CoA also covered *Cronobacter sakazakii*.

Samples of all shipped iLiNS products are held at Nutriset at two temperatures (30 and 40°C). In addition to analyses performed before shipment, annual analyses are carried out on all products, including complete analysis of vitamins and minerals, radioactivity (cesium 134 and 137), heavy metals and dioxin. In addition, as noted earlier, products are tested for organoleptic characteristics.

Aflatoxin

Peanut is a common ingredient in LNS, including the iLiNS Project formulations. Peanut – along with other legumes and grains – may be vulnerable to contamination with aflatoxin, a potentially carcinogenic mycotoxin produced by a fungus (*Aspergillus*). Contamination is most prevalent in tropical areas and/or where climatic conditions (drought) or storage for raw ingredients are inadequate. In these environments, other staple foods, notably maize, are also commonly contaminated. Aflatoxin levels in these foods can vary from a few parts per billion (ppb) up to thousands of ppb (Food and Agriculture Organization 2004). As chronic consumption may lead to stunted growth in children (Khlangwiset *et al.* 2011), it is particularly important to manage risk of aflatoxin contamination in products designed for infants. As noted earlier, total aflatoxins are routinely tested, both in peanuts and in iLiNS supplements,

with a tolerance level of 4 ppb in peanuts (European regulation) and 5 ppb in iLiNS supplements (Codex Standard 193–1995).

Peanut allergy

Peanut allergy is a high-profile problem in the United States and some other high-income countries, with prevalence reported to be increasing (Burks 2008). Among infants and young children under 2 years of age in the United States, the most common allergies are to milk (2%), peanut (1.4%) and egg (1.0%) followed by strawberries, shellfish, wheat, soy, fin fish and tree nuts. For affected children, the foods associated with the highest likelihood of severe reactions are peanut and tree nuts at 52% of those affected (or 0.7% of all children), compared with about 30% for milk (0.6% of all children) and for egg (0.3% of all children) (Gupta *et al*. 2011).

Globally, there is little information on the prevalence of peanut or other allergies in low-income countries, but prevalence is thought to be lower than in developed countries. The prevalence of peanut allergy in Africa is unknown (Yang 2010). Prevalence of other food allergies common in developed countries (milk, eggs and seafood) is also unknown.

Anecdotally, researchers who have been involved with previous studies of peanut-based RUTF and LNS in Africa report that no adverse allergic reactions have been seen during observation of thousands of test doses (Yang 2010). Further, a recent systematic review concluded that there is no clear evidence that either maternal exposure or timing of introduction of peanuts into infant and toddler diets has an impact on later development of allergy (Thompson *et al*. 2010).

Currently, both the American Academy of Pediatrics and the National Institute of Allergy and Infectious Diseases advise no avoidance of potential allergens during pregnancy or lactation. They also advise no general restriction on introduction of potentially allergenic foods in infancy (after 6 months of age, when complementary feeding is recommended to commence) (Burks *et al*. 2011; Fleischer *et al*. 2013).

Box 7. Key considerations for packaging of LNS

- Product packaging choices are determined by (and affect) product quality and shelf life, cost considerations, delivery logistics, convenience for consumers, environmental impact, and the need to communicate with many audiences (customs officials, programme staff and/or vendors, consumers).
- 'Primary packaging' is the layer of packaging in direct contact with the LNS (vs. cartons, etc.); the two options for primary packaging are sachets or cups.
- Single-serving sachets are most convenient for consumers and may simplify communication with consumers regarding appropriate dosage.
- Sachets have a longer shelf life than cups.
- Sachets can be packed more tightly and can therefore reduce transportation costs.
- Currently, sachets cannot be recycled or repurposed, but cups can be either recycled or reused.
- iLiNS Project studies have used both sachets and cups. Both were well-accepted by study participants. Project experiences confirm challenges with standardising a daily dose when using cups.

Summary of safety and risks of iLiNS supplements

In choosing ingredients for the iLiNS formulations, all known risks were considered. In our research context, risks from aflatoxin, chemical and microbial contamination are minimised by purchase of the iLiNS supplements from a supplier with high-quality standards and procedures (Nutriset-France). Risk of allergic reactions remains but is judged to be no higher than the allergy risk associated with providing cow's milk; providing infants with supplements that include milk as an ingredient has been widely carried out and considered acceptable in the past in both research and public health practice.

Considerations for packaging

This section provides a general overview of considerations and choices related to packaging, and concludes with a description of iLiNS Project choices and experiences (Box 7).

The packaging of LNS products has several purposes:

• protect and conserve the product (quality assurance, shelf life and prevention of leakage from the package);

• facilitate transportation, storage and distribution (logistics);
• inform the consumer and those handling the product (graphic design, legal, regulatory and marketing aspects); and
• minimise negative impact on the environment through use of materials that can be recycled or are biodegradable or can be composted.

Several packaging levels (primary, secondary and tertiary) need to be considered to optimise production, storage, transportation, distribution and consumption in terms of price, quality and convenience.

The primary packaging contains, and is in direct contact with, the product. The main task of the primary packaging is to protect the lipid-rich product from oxidation and contamination that could degrade product quality. The packaging material therefore must be appropriate for packaging of food products and provide a sufficient barrier for oxygen, light and moisture to preserve product quality throughout the intended shelf life.

Most packaging options for food products would be possible for LNS products, but considering costs and the possibility of local production, the choice quickly comes down to cups or sachets. Cups are probably the easiest option where packaging is carried out manually. Once production is scaled-up through automation, sachets may be preferred because they provide the longest shelf life at the lowest cost (material and logistics).

Barrier protection, product stability and disposal of primary packaging

The sachet material usually consists of multiple layers and each fulfils a specific purpose. The appropriate foil for LNS products has an inner layer adapted for an oily product that ensures a resistant seal, followed by a thin metalised film constituting a barrier for oxygen, moisture and UV transmission and often an additional outer layer. The printing process must be designed so that the ink cannot be ingested if the product is eaten directly from the sachet. Sachet packaging can take place in a low oxygen atmosphere to further reduce oxidation reactions of the product. These packaging properties provide a basis for a shelf life of up to 24 months.

The cup consists of several components; a plastic cup is made of polypropylene, an airtight seal to increase shelf life and a lid to protect the seal and to cover the cup after the seal has been removed. As it is difficult to fill a cup in a low oxygen atmosphere, the shelf life of LNS in a cup is shorter than when packaged in a multilayer sachet.

The material cost of a sachet is about half of the material cost of a cup. But because each component of the cup is made of a single material, the different components could be recycled. The empty cup is also often appreciated by the consumer who can reuse it as a container. The empty sachet cannot be directly reused and separating the different layers of the sachet foil for recycling is of no economic interest. The foil material could be transformed to make furniture or other items as the material is robust, but collection of the empty sachets is a logistical challenge that involves a cost. Research is ongoing to determine possibilities to modify the sachets and make them reusable without transformation of the material (e.g. to create a cooling box out of a carton box and the foil of the empty sachets). The long-term objective is to find a packaging material that is biodegradable and still provides adequate shelf life.

Convenience, portion control and logistics

Single-serving sizes of LNS can vary considerably between product type, target group and context. The size of the sachet is very flexible and can easily be adapted to the required single-serving size or adjusted to contain multiple servings. Packaging units can be as small as 10 g or greater than 150 g. While a single-serving size usually increases the product's convenience, the bigger the packaging unit, the faster large amounts can be manufactured, thereby reducing product costs.

Because LNS have a very low water activity, bacterial growth is not a risk before or after opening the packaging (assuming water content is not altered). Reclosing the packaging can prevent contamination

of the product with insects or dirt. The possibility of reclosing the cup with a lid makes it more convenient for the provision of multiple servings in one packaging unit. Simple technologies are available to reseal a foil sachet, but they would increase production costs and/or costs for including an additional educational component during distribution in order to explain the resealing mechanism.

Without a resealing option, the sachet loses its convenience if the portion size does not correspond to a single serving or is not consumed all at once. Mothers who participated in an acceptability study in Niger in 2009 reported that they did not like having to split the contents of a single 20-g sachet into two servings per day (Tripp *et al*. 2011). They felt it was difficult to correctly serve the child half the contents, and they felt it was unhygienic to leave the sachet open throughout the day.

Multiple-serving packaging may also increase household sharing because the opened packaging 'invites' immediate consumption. Once opened, the product may be shared with others to finish it as soon as possible out of fear that the product could spoil and would be wasted.

Single-serving packaging units are more convenient for the recipient and could improve correct usage, but the smaller the packaging unit, the more units need to be distributed per person. A weekly ration of daily doses involves 7 LNS sachets, or 14 sachets if the LNS is intended to be consumed in two separate servings per day. To distribute a monthly ration, 30 or 60 sachets would need to be counted during distribution and would require secondary packaging of some kind.

Regrouping single servings into a weekly or monthly ration package facilitates distribution by reducing time needed to count and hand out the primary packages. Secondary bags to regroup a monthly ration of 30 and 60 single servings for children and women were used in Guatemala in 2011. Hard plastic containers used to store the sachets at home (one per household) are being distributed in the Rang-Din Nutrition Study, Bangladesh.

Secondary packaging adds additional packaging costs and an additional repackaging step. Alternatively, sachets can be produced in strips of multiple single sachets, so that regrouping them involves no extra costs or additional packaging material. The number of sachets in a strip should be convenient; strips of seven sachets provide a weekly ration.

Tertiary packaging is necessary to provide physical protection of the product during transportation and to facilitate storage/stacking and handling. A standardised carton box that is adapted to international pallet sizes maximises the quantities that fit onto a pallet and into a container. Carton boxes that are recyclable and biodegradable are available and are not an environmental concern.

Sachets can be packed more efficiently than cups ('packaging efficiency' is the volume of space occupied by the product/total volume). The more product that can fit into a box, and consequently into a container, the lower the transportation costs per net weight. The smaller the packaging unit, the more space is taken up by the packaging material, thereby decreasing packaging efficiency. Thus, the larger the sachet, the higher the packaging efficiency.

Information transmission and marketing

The labelling of the product should comply with the 'General Standard for the Labeling of prepackaged foods' (CODEX STAN 1-1985).

Mandatory information on the primary packaging includes:

- brand name/ trade name;
- name, location and address of the manufacturer; country of origin where applicable;
- ingredients in descending order of proportions (by weight);
- date of manufacture;
- best before/ expiry date;
- batch/lot number to trace the product and its ingredients;
- net mass (in metric units);
- conditions required for storage; and
- directions for use.

Any additional information that is made mandatory for LNS products could be displayed on other packaging components (secondary or tertiary packaging)

or on a technical data sheet or tag if the space on the primary packaging cannot accommodate the information. An increase in mandatory information on product packaging can have significant cost implications if the information cannot be added to the print of existing packaging material and, thus, implies additional material and manufacturing costs.

If space allows, additional marking is possible in accordance with relevant Codex Alimentarius standards and national legislation.

According to the Codex general guidelines on claims (CAC/GL 1-1979) and guidelines for use of nutrition and health claims (CAC/GL 23-1997), claims need to be consistent with the national nutrition policy and should not be misleading.

LNS products targeted at infants and young children and the related communication should also comply with the International Code of Marketing of Breastmilk Substitutes and subsequent relevant resolutions of the World Health Assembly and should support optimal infant feeding. The Working Paper *Using the Code of Marketing of Breast-milk Substitutes to Guide the Marketing of Complementary Foods to Protect Optimal Infant Feeding Practices* (Quinn *et al*. 2010) provides provisional guidance on labelling.

To encourage proper use for breastfed children, labelling should:

- be easy to read (with information being visible before purchase);
- include instructions/messages written in a local language;
- specify the appropriate age for the introduction of the supplement;
- emphasise the importance of exclusive breastfeeding for the first 6 months of life;
- encourage continued breastfeeding up to 2 years and beyond;
- provide instructions for safe and appropriate preparation, use and storage of the supplement;
- state the daily serving size, which should not exceed the recommended energy intake from complementary foods for breastfed children; and
- not include pictures of babies appearing to be younger than 6 months.

Additional information or pictorial figures may be displayed on the packaging provided that it is not in conflict with the mandatory requirements of the general standard and those relating to claims.

iLiNS Project LNS packaging and messages

Decisions on packaging of iLiNS supplements followed from earlier successful experiences in two of the three field sites. Based on their prior experiences, the Malawi site team initially requested that Nutriset package the supplement in cups while the Ghana site team requested sachets. In Burkina Faso, the team initially chose cups following the lead of the Malawi team. Later, it became clear that sachets would be less costly and have a longer shelf life. Subsequently, the Burkina site switched to sachets, and the Malawi site switched to sachets for a second study. This also reflected the Malawi team's experience with the complications of communicating to study subjects how to provide infants with the correct daily dose when using cups. Also, costs for use of cups in Malawi were increased by the decision to provide study households with standardised spoons to help ensure correct dosing.

Product packaging for the iLiNS studies was not used to convey messages on infant feeding nor on use of the LNS. Instead, a set of basic messages was delivered to participants upon enrolment. Messages varied slightly by study site, but followed the same general approach. The approach was to deliver a relatively small number of messages, both to avoid overwhelming the participants with information, and to mimic the amount of information that might be conveyed through the health system in the local sites. The intent was to help ensure that the LNS was consumed by the targeted person in the correct dose and to convey that LNS should not displace breast milk or local foods. Simple messages were also developed for women who received LNS during pregnancy and lactation. For example, for infants receiving LNS, the following generic messages were developed and then adapted and translated in each site, with 'LNS' replaced by the local name:

1. The LNS is all for [NAME] because babies need special foods at X–X months [age range depends on objective/trial].
2. Breastfeed your child just as you did before we gave you LNS.
3. Give your baby meat, fish, eggs, fruits and vegetables whenever you can. Babies need these foods even if they eat the LNS [add milk/dairy to list if available].
4. Give your baby X spoons/sachets of LNS each day. It is better to give it twice, X spoons/sachets each time.
5. Do not give more than X spoons/sachets each day because it is not good for the baby to have too much.
6. It is best if you mix the LNS with just a little bit of porridge [2–3 large spoonfuls (or appropriate household measure)] and feed the mixture to the baby before feeding the rest of the porridge.
7. Store the LNS someplace where it will stay dry and away from children. Store in the coolest dry place that you can find in your house.

Summary of considerations for developing LNS for prevention of undernutrition

The iLiNS Project aims to contribute to knowledge about the potential usefulness of LNS for prevention of undernutrition among vulnerable groups of infants, young children, and pregnant and lactating women. While our efficacy trials are ongoing and results are not yet available, we recognise that interest in LNS for a range of applications is growing, and that the development of new products by a variety of actors is also ongoing. This paper presents the rationale for decisions taken while developing the LNS for our project.

In doing so, we highlight the difference between small-quantity LNS as a vehicle for home fortification in contrast to medium-quantity LNS, which provide a more substantial proportion of energy needs. Medium-quantity LNS may exceed the energy needs from food of breastfed infants 6–8 months of age, leaving little to no room for diversifying diets. It is also possible that medium-quantity LNS may exceed infant appetite and increase the likelihood of the supplement being shared, and of not delivering the desired micronutrients and EFA to the target child. To date, available information suggests that – compared to providing FBF – providing LNS in 25–50-g doses does *not* displace breast milk intake; but more studies are warranted, as are more studies assessing impact (if any) of varying quantities of LNS on dietary diversification, and on the likelihood of sharing/leakage.

Our review of considerations for selecting ingredients suggests that a variety of LNS formulations are palatable and acceptable to children and their mothers. Choice of ingredients can determine EFA content and can also result in varying quantities/concentrations of anti-nutrients, which should be considered when setting micronutrient levels in the LNS. Whether or not small quantities of milk contribute to the efficacy of LNS for promoting growth remains unknown, and is under study in our project.

The micronutrient and EFA contents of our supplements for infants/young children were generally chosen to provide approximately one RDA or RNI for most micronutrients. Iron content was set considering current knowledge about risks (World Health Organization 2007). Because the age range of target children spans two different age categories in the requirements, choices were made based on assumptions about nutrients likely to be provided, e.g. by breast milk. We note that the LNS, unlike MNP, provide several macrominerals needed for growth, as well as EFA. Up to now, MNP have not been shown to promote linear growth (De-Regil *et al*. 2011), whereas results from two studies suggested an impact of LNS on growth or stunting (Adu-Afarwuah *et al*. 2007b; Phuka *et al*. 2008, 2009b). Our iLiNS trials aim to re-examine these results and extend observations in several settings.

The approach for setting nutrient levels for pregnant and lactating women was slightly different in that the iLiNS formulation was influenced by a recent study showing better results with an antenatal supplement providing micronutrients (except iron) at twice the RDA/RNI (Kaestel *et al*. 2005). Our supplements do the same, with several exceptions due to considerations of safety (vitamin A, folic acid and iron) or feasibility (vitamin C) or in harmony with a newer

recommendation (iodine). We also provide additional micronutrients not found in the UNIMMAP supplements as well as EFA.

Our summary of considerations related to safety of ingredients and products – while not unique to LNS – highlights the many challenges faced by producers. Available international standards provide a framework. Finally, iLiNS Project researchers and partners now have experience with several different packaging options for LNS. We hope that the discussion of the advantages and disadvantages of the two main options (cups and sachets) can inform choices made by others.

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Conflicts of interest

One author (MZ) is employed by Nutriset S.A.S., the company that developed and produced LNS for the iLiNS Project, and another author (SJ) was previously employed by Nutriset. All other authors declare no conflicts of interest.

Contributions

KGD, PA, MZ, KHB and LHA participated in the discussions and decision making about the supplements that form the basis for the paper. MA, KGD and MZ conceptualised the paper. MA, MZ, SJ and KGD wrote the paper. All authors reviewed and provided substantive contributions to drafts. All authors read and approved the final draft.

References

Ackatia-Armah R.S., McDonald C., Doumbia S. & Brown K.H. (2012) Effect of selected dietary regimens on recovery from moderate acute malnutrition in young Malian children. *FASEB Journal* **26**, 1031.10.

Adu-Afarwuah S., Lartey A., Brown K.H., Zlotkin S., Briend A. & Dewey K.G. (2007a) A fat-based supplement containing essential fatty acids increased plasma-linolenic acid and linear growth of Ghanaian infants. *FASEB Journal* **21**, 223.7.

Adu-Afarwuah S., Lartey A., Brown K.H., Zlotkin S., Briend A. & Dewey K.G. (2007b) Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on growth and motor development. *American Journal of Clinical Nutrition* **86**, 412–420.

Adu-Afarwuah S., Lartey A., Brown K.H., Zlotkin S., Briend A. & Dewey K.G. (2008) Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. *American Journal of Clinical Nutrition* **87**, 929–938.

Adu-Afarwuah S., Lartey A., Zeilani M. & Dewey K.G. (2011) Acceptability of lipid-based nutrient supplements (LNS) among Ghanaian infants and pregnant or lactating women. *Maternal and Child Nutrition* **7**, 344–356.

Allen L., de Benoist B., Dary O. & Hurrell R. (eds) (2006) *WHO Guidelines on Food Fortification with Micronutrients*. World Health Organization: Geneva.

Arimond M., Wiesmann D., Becquey E., Carriquiry A., Daniels M.C., Deitchler M. *et al*. (2010) Simple food group diversity indicators predict micronutrient adequacy of women's diets in 5 diverse, resource-poor settings. *Journal of Nutrition* **140**, 2059S–2069S.

Barrett J.F., Whittaker P.G., Williams J.G. & Lind T. (1994) Absorption of non-haem iron from food during normal pregnancy. *BMJ* **309**, 79–82.

Beauchamp G.K. & Mennella J.A. (2011) Flavor perception in human infants: development and functional significance. *Digestion* **83** (Suppl. 1), 1–6.

Bisimwa G., Owino V.O., Bahwere P., Dramaix M., Donnen P., Dibari F. *et al*. (2012) Randomized controlled

trial of the effectiveness of a soybean-maize-sorghum-based ready-to-use complementary food paste on infant growth in South Kivu, Democratic Republic of Congo. *American Journal of Clinical Nutrition* **95**, 1157–1164.

Burks A.W. (2008) Peanut allergy. *Lancet* **371**, 1538–1546.

Burks A.W., Jones S.M., Boyce J.A., Sicherer S.H., Wood R.A., Assa'ad A. *et al*. (2011) NIAID-sponsored 2010 guidelines for managing food allergy: applications in the pediatric population. *Pediatrics* **128**, 955–965.

Chaparro C.M. & Dewey K.G. (2010) Use of lipid-based nutrient supplements (LNS) to improve the nutrient adequacy of general food distribution rations for vulnerable sub-groups in emergency settings. *Maternal and Child Nutrition* **6** (Suppl. 1), 1–69.

Christian P., Jiang T., Khatry S.K., LeClerq S.C., Shrestha S.R. & West K.P. Jr (2006) Antenatal supplementation with micronutrients and biochemical indicators of status and subclinical infection in rural Nepal. *American Journal of Clinical Nutrition* **83**, 788–794.

Ciliberto M.A., Sandige H., Ndekha M.J., Ashorn P., Briend A., Ciliberto H.M. *et al*. (2005) Comparison of home-based therapy with ready-to-use therapeutic food with standard therapy in the treatment of malnourished Malawian children: a controlled, clinical effectiveness trial. *American Journal of Clinical Nutrition* **81**, 864–870.

Cohuet S., Marquer C., Shepherd S., Captier V., Langendorf C., Ale F. *et al*. (2012) Intra-household use and acceptability of ready-to-use-supplementary-foods distributed in Niger between July and December 2010. *Appetite* **59**, 698–705.

De-Regil L.M., Suchdev P.S., Vist G.E., Walleser S. & Pena-Rosas J.P. (2011) Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database of Systematic Reviews* (9), CD008959.

Devaney B. & Fox M.K. (2008) Dietary intakes of infants and toddlers: problems start early. In: *Eating Behaviors of the Young Child: Prenatal and Postnatal Influences on Healthy Eating* (eds L. Birch & W. Dietz), pp 123–140. American Academy of Pediatrics: Elk Grove Village, IL.

Dewey K.G. & Arimond M. (2012) Lipid-based nutrient supplements: how can they combat child malnutrition? *PLoS Medicine*. doi: 10.1371/journal.pmed.1001314.

Dewey K.G. & Brown K.H. (2003) Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin* **24**, 5–28.

Dewey K.G. & Mayers D.R. (2011) Early child growth: how do nutrition and infection interact? *Maternal and Child Nutrition* **7** (Suppl. 3), 129–142.

Diop el H.I., Dossou N.I., Ndour M.M., Briend A. & Wade S. (2003) Comparison of the efficacy of a solid ready-to-use food and a liquid, milk-based diet for the rehabilitation of severely malnourished children: a randomized trial. *Am J Clin Nutr* **78**, 302–307.

Dror D.K. & Allen L.H. (2011) The importance of milk and other animal-source foods for children in low-income countries. *Food and Nutrition Bulletin* **32**, 227–243.

Fawzi W.W., Msamanga G.I., Spiegelman D., Urassa E.J., McGrath N., Mwakagile D. *et al*. (1998) Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet* **351**, 1477–1482.

Flax V.L., Ashorn U., Phuka J., Maleta K., Manary M.J. & Ashorn P. (2008) Feeding patterns of underweight children in rural Malawi given supplementary fortified spread at home. *Maternal and Child Nutrition* **4**, 65–73.

Flax V.L., Thakwalakwa C., Phuka J., Ashorn U., Cheung Y.B., Maleta K. *et al*. (2009) Malawian mothers' attitudes towards the use of two supplementary foods for moderately malnourished children. *Appetite* **53**, 195–202.

Flax V.L., Phuka J., Cheung Y.B., Ashorn U., Maleta K. & Ashorn P. (2010) Feeding patterns and behaviors during home supplementation of underweight Malawian children with lipid-based nutrient supplements or corn-soy blend. *Appetite* **54**, 504–511.

Fleischer D.M., Spergel J.M., Assa'ad A.H. & Pongracic J.A. (2013) Primary prevention of allergic disease through nutritional interventions. *Journal of Allergy and Clinical Immunology* **1**, 29–36.

Food and Agriculture Organization (2004) *Worldwide Regulations for Mycotoxins in Food and Feed*. Food and Agriculture Organization: Rome.

Food and Agriculture Organization (2010) *Fats and Fatty Acids in Human Nutrition: Report of an Expert Consultation. FAO Food and Nutrition Paper 91*. Food and Agriculture Organization: Rome.

Galpin L., Thakwalakwa C., Phuka J., Ashorn P., Maleta K., Wong W.W. *et al*. (2007) Breast milk intake is not reduced more by the introduction of energy dense complementary food than by typical infant porridge. *Journal of Nutrition* **137**, 1828–1833.

Gibson R.S. & Hotz C. (2000) The adequacy of micronutrients in complementary foods. *Pediatrics* **106**, 1298–1299.

Gibson R.S., Bailey K.B., Gibbs M. & Ferguson E.L. (2010) A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin* **31**, S134–S146.

Global Nutrition Cluster (2012) *Moderate Acute Malnutrition: A Decision Tool for Emergencies*. Available at: http://www.unicef.org/nutritioncluster/files/MAM_DecisionTool_July_2012_with_Cover.pdf (Accessed 22 March 2013).

Golden M.H. (1995) Specific deficiencies versus growth failure: type I and type II nutrients. *SCN News* **12**, 15–21.

Golden M.H. (2009) Proposed recommended nutrient densities for moderately malnourished children. *Food and Nutrition Bulletin* **30**, S267–S342.

Grellety E., Shepherd S., Roederer T., Manzo M.L., Doyon S., Ategbo E.A. *et al*. (2012) Effect of mass supplementation with ready-to-use supplementary food during an anticipated nutritional emergency. *PLoS ONE*. doi: 10.1371/journal.pone.0044549.

Gupta R.S., Springston E.E., Warrier M.R., Smith B., Kumar R., Pongracic J. *et al*. (2011) The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics* **128**, e9–e17.

Heidkamp R.A., Stoltzfus R.J., Fitzgerald D.W. & Pape J.W. (2012) Growth in late infancy among HIV-exposed children in urban Haiti is associated with participation in a clinic-based infant feeding support intervention. *Journal of Nutrition* **142**, 774–780.

Hess S.Y., Bado L., Aaron G.J., Ouedraogo J.B., Zeilani M. & Brown K.H. (2011) Acceptability of zinc-fortified, lipid-based nutrient supplements (LNS) prepared for young children in Burkina Faso. *Maternal and Child Nutrition* **7**, 357–367.

Home Fortification Technical Advisory Group (2011) *Programmatic Guidance Brief on Use of Micronutrient Powders (MNP) for Home Fortification*. Available at: http://hftag.gainhealth.org/resources/programmatic-guidance-brief-use-micronutrient-powders-mnp-home-fortification (Accessed 3 December 2012).

Hoppe C., Molgaard C. & Michaelsen K.F. (2006) Cow's milk and linear growth in industrialized and developing countries. *Annual Review of Nutrition* **26**, 131–173.

Huffman S.L., Harika R.K., Eilander A. & Osendarp S.J. (2011) Essential fats: how do they affect growth and development of infants and young children in developing countries? A literature review. *Maternal and Child Nutrition* **7** (Suppl. 3), 44–65.

Humphrey J.H., Iliff P.J., Marinda E.T., Mutasa K., Moulton L.H., Chidawanyika H. *et al*. (2006) Effects of a single large dose of vitamin A, given during the postpartum period to HIV-positive women and their infants, on child HIV infection, HIV-free survival, and mortality. *Journal of Infectious Diseases* **193**, 860–871.

Huybregts L., Roberfroid D., Lanou H., Menten J., Meda N., Van Camp J. *et al*. (2009) Prenatal food supplementation fortified with multiple micronutrients increases birth length: a randomized controlled trial in rural Burkina Faso. *American Journal of Clinical Nutrition* **90**, 1593–1600.

Huybregts L., Houngbe F., Salpeteur C., Brown R., Roberfroid D., Ait-Aissa M. *et al*. (2012) The effect of adding ready-to-use supplementary food to a general food distribution on child nutritional status and morbidity: a cluster-randomized controlled trial. *PLoS Medicine*. doi: 10.1371/journal.pmed.1001313.

Ickes S.B., Jilcott S.B., Myhre J.A., Adair L.S., Thirumurthy H., Handa S. *et al*. (2012) Examination of facilitators and barriers to home-based supplemental feeding with ready-to-use food for underweight children in western Uganda. *Maternal and Child Nutrition* **8**, 115–129.

Innis S.M. (2008) Dietary omega 3 fatty acids and the developing brain. *Brain Research* **1237**, 35–43.

Institute of Medicine (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*. National Academies Press: Washington, DC.

Isanaka S., Nombela N., Djibo A., Poupard M., Van Beckhoven D., Gaboulaud V. *et al*. (2009) Effect of preventive supplementation with ready-to-use therapeutic food on the nutritional status, mortality, and morbidity of children aged 6 to 60 months in Niger: a cluster randomized trial. *JAMA* **301**, 277–285.

Isanaka S., Roederer T., Djibo A., Luquero F.J., Nombela N., Guerin P.J. *et al*. (2010) Reducing wasting in young children with preventive supplementation: a cohort study in Niger. *Pediatrics* **126**, e442–e450.

Kaestel P., Michaelsen K.F., Aaby P. & Friis H. (2005) Effects of prenatal multimicronutrient supplements on birth weight and perinatal mortality: a randomised, controlled trial in Guinea-Bissau. *European Journal of Clinical Nutrition* **59**, 1081–1089.

Khlangwiset P., Shephard G.S. & Wu F. (2011) Aflatoxins and growth impairment: a review. *Critical Reviews in Toxicology* **41**, 740–755.

Kuusipalo H., Maleta K., Briend A., Manary M. & Ashorn P. (2006) Growth and change in blood haemoglobin concentration among underweight Malawian infants receiving fortified spreads for 12 weeks: a preliminary trial. *Journal of Pediatric Gastroenterology and Nutrition* **43**, 525–532.

Labuza T.P., Warren R.M. & Warmbier H.C. (1977) The physical aspects with respect to water and non-enzymatic browning. *Advances in Experimental Medicine and Biology* **86B**, 379–418.

Lagrone L., Cole S., Schondelmeyer A., Maleta K. & Manary M.J. (2010) Locally produced ready-to-use supplementary food is an effective treatment of moderate acute malnutrition in an operational setting. *Annals of Tropical Paediatrics* **30**, 103–108.

LaGrone L.N., Trehan I., Meuli G.J., Wang R.J., Thakwalakwa C., Maleta K. *et al*. (2012) A novel fortified blended flour, corn-soy blend 'plus-plus,' is not inferior to lipid-based ready-to-use supplementary foods for the treatment of moderate acute malnutrition in Malawian

children. *American Journal of Clinical Nutrition* **95**, 212–219.

Lauritzen L. & Carlson S.E. (2011) Maternal fatty acid status during pregnancy and lactation and relation to newborn and infant status. *Maternal and Child Nutrition* **7** (Suppl. 2), 41–58.

Lee S.E., Talegawkar S.A., Merialdi M. & Caulfield L.E. (2012) Dietary intakes of women during pregnancy in low- and middle-income countries. *Public Health Nutrition*. [Epub ahead of print].

Lin C.A., Manary M.J., Maleta K., Briend A. & Ashorn P. (2008) An energy-dense complementary food is associated with a modest increase in weight gain when compared with a fortified porridge in Malawian children aged 6–18 months. *Journal of Nutrition* **138**, 593–598.

Macé K., Shahkhalili Y., Aprikian O. & Stan S. (2006) Dietary fat and fat types as early determinants of childhood obesity: a reappraisal. *International Journal of Obesity (2005)* **30** (Suppl. 4), S50–S57.

Makrides M., Collins C.T. & Gibson R.A. (2011) Impact of fatty acid status on growth and neurobehavioural development in humans. *Maternal and Child Nutrition* **7** (Suppl. 2), 80–88.

Margetts B.M., Fall C.H., Ronsmans C., Allen L.H. & Fisher D.J. (2009) Multiple micronutrient supplementation during pregnancy in low-income countries: review of methods and characteristics of studies included in the meta-analyses. *Food and Nutrition Bulletin* **30**, S517–S526.

Matias S.L., Chaparro C.M., Perez-Exposito A.B., Peerson J.M. & Dewey K.G. (2011) Acceptability of a lipid-based nutrient supplement among Guatemalan infants and young children. Washington, DC: FHI 360/FANTA-2 Bridge.

Matilsky D.K., Maleta K., Castleman T. & Manary M.J. (2009) Supplementary feeding with fortified spreads results in higher recovery rates than with a corn/soy blend in moderately wasted children. *Journal of Nutrition* **139**, 773–778.

Michaelsen K.F., Hoppe C., Roos N., Kaestel P., Stougaard M., Lauritzen L. *et al.* (2009) Choice of foods and ingredients for moderately malnourished children 6 months to 5 years of age. *Food and Nutrition Bulletin* **30**, S343–S404.

Michaelsen K.F., Dewey K.G., Perez-Exposito A.B., Nurhasan M., Lauritzen L. & Roos N. (2011) Food sources and intake of n-6 and n-3 fatty acids in low-income countries with emphasis on infants, young children (6–24 months), and pregnant and lactating women. *Maternal and Child Nutrition* **7** (Suppl. 2), 124–140.

Milman N., Byg K.E., Bergholt T., Eriksen L. & Hvas A.M. (2006) Body iron and individual iron prophylaxis in pregnancy – should the iron dose be adjusted according to serum ferritin? *Annals of Hematology* **85**, 567–573.

Mølgaard C., Larnkjaer A., Arnberg K. & Michaelsen K.F. (2011) Milk and growth in children: effects of whey and casein. *Nestle Nutrition Workshop Series. Paediatric Programme* **67**, 67–78.

Mridha M.K., Chaparro C.M., Matias S.L., Hussain S., Munira S., Saha S. *et al.* (2012) Acceptability of lipid-based nutrient supplements and micronutrient powders among pregnant and lactating women and infants and young children in Bangladesh and their perceptions about malnutrition and nutrient supplements. Washington, DC: FHI 360/FANTA-2 Bridge.

Nackers F., Broillet F., Oumarou D., Djibo A., Gaboulaud V., Guerin P.J. *et al.* (2010) Effectiveness of ready-to-use therapeutic food compared to a corn/soy-blend-based pre-mix for the treatment of childhood moderate acute malnutrition in Niger. *Journal of Tropical Pediatrics* **56**, 407–413.

Ndekha M. (1998) *The Dietary Intake of Pregnant Women in Rural Malawi – A Cohort Study*. MSc Thesis, Uppsala University: Uppsala, Sweden.

Nicklaus S. (2009) Development of food variety in children. *Appetite* **52**, 253–255.

Otten J.J., Hellwig J.P. & Meyers L.D. (eds) (2006) *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. National Academies Press: Washington, DC.

Owino V.O., Bahwere P., Bisimwa G., Mwangi C.M. & Collins S. (2011) Breast-milk intake of 9–10-mo-old rural infants given a ready-to-use complementary food in South Kivu, Democratic Republic of Congo. *American Journal of Clinical Nutrition* **93**, 1300–1304.

Pan American Health Organization/World Health Organization (2003) Guiding Principles for Complementary Feeding of the Breastfed Child. Pan American Health Organization: Washington, DC.

Parker M.E., Bentley M.E., Chasela C., Adair L., Piwoz E.G., Jamieson D.J. *et al.* (2011) The acceptance and feasibility of replacement feeding at 6 months as an HIV prevention method in Lilongwe, Malawi: results from the BAN study. *AIDS Education and Prevention* **23**, 281–295.

Paul K.H., Muti M., Chasekwa B., Mbuya M.N., Madzima R.C., Humphrey J.H. *et al.* (2012) Complementary feeding messages that target cultural barriers enhance both the use of lipid-based nutrient supplements and underlying feeding practices to improve infant diets in rural Zimbabwe. *Maternal and Child Nutrition* **8**, 225–238.

Phuka J., Maleta K., Thakwalakwa C., Cheung Y.B., Briend A., Manary M.J. *et al.* (2008) Complementary feeding

with fortified spread and incidence of severe stunting in 6- to 18-month-old rural Malawians. *Archives of Pediatrics and Adolescent Medicine* **162**, 619–626.

Phuka J., Thakwalakwa C., Maleta K., Cheung Y.B., Briend A., Manary M. *et al*. (2009a) Supplementary feeding with fortified spread among moderately underweight 6–18-month-old rural Malawian children. *Maternal and Child Nutrition* **5**, 159–170.

Phuka J., Maleta K., Thakwalakwa C., Cheung Y.B., Briend A., Manary M.J. *et al*. (2009b) Postintervention growth of Malawian children who received 12-mo dietary complementation with a lipid-based nutrient supplement or maize-soy flour. *American Journal of Clinical Nutrition* **89**, 382–390.

Phuka J., Ashorn U., Ashorn P., Zeilani M., Cheung Y.B., Dewey K.G. *et al*. (2011) Acceptability of three novel lipid-based nutrient supplements among Malawian infants and their caregivers. *Maternal and Child Nutrition* **7**, 368–377.

Piwoz E.G., Huffman S.L. & Quinn V.J. (2003) Promotion and advocacy for improved complementary feeding: can we apply the lessons learned from breastfeeding? *Food and Nutrition Bulletin* **24**, 29–44.

Piwoz E.G., Sundberg S. & Rooke J. (2012) Promoting healthy growth: what are the priorities for research and action? *Advances in Nutrition* **3**, 234–241.

Quinn V.J., Zehner E., Schofield D., Guyon A.B. & Huffman S.L. (2010) Using the Code of Marketing of Breast-milk Substitutes to Guide the Marketing of Complementary Foods to Protect Optimal Infant Feeding Practices. Global Alliance for Improved Nutrition: Geneva.

Rolland-Cachera M.F., Maillot M., Deheeger M., Souberbielle J.C., Peneau S. & Hercberg S. (2012) Association of nutrition in early life with body fat and serum leptin at adult age. *International Journal of Obesity*. doi: 10.1038/ijo.2012.185.

Roos N., Sorensen J.C., Sorensen H., Rasmussen S.K., Briend A., Yang Z. *et al*. (2013) Screening for anti-nutritional compounds in complementary foods and food aid products for infants and young children. *Maternal and Child Nutrition* **9** (Suppl. 1), 47–71.

Talley L., Boyd E., el Sharief F., Blanton C., OmerAli M. & Abd el Rahman M. (2012) Prevention of acute malnutrition during the lean season: comparison of a lipid-based nutrient supplement and an improved dry ration, South Darfur, Sudan. A quasi-experimental study. *Journal of Nutritional Disorders and Therapy*. doi: 10.4172/2161-0509.1000117.

Ten Year Strategy to Reduce Vitamin and Mineral Deficiencies, Maternal, Infant, and Young Child Nutrition Working Group: Formulation Subgroup (2009) Formulations for fortified complementary foods and supplements: review of successful products for improving the nutritional status of infants and young children. *Food and Nutrition Bulletin* **30**, S239–S255.

Thakwalakwa C., Ashorn P., Phuka J., Cheung Y.B., Briend A., Puumalainen T. *et al*. (2010) A lipid-based nutrient supplement but not corn-soy blend modestly increases weight gain among 6- to 18-month-old moderately underweight children in rural Malawi. *Journal of Nutrition* **140**, 2008–2013.

Thakwalakwa C.M., Ashorn P., Jawati M., Phuka J.C., Cheung Y.B. & Maleta K.M. (2012) An effectiveness trial showed lipid-based nutrient supplementation but not corn-soya blend offered a modest benefit in weight gain among 6- to 18-month-old underweight children in rural Malawi. *Public Health Nutrition* **15**, 1755–1762.

Thompson R.L., Miles L.M., Lunn J., Devereux G., Dearman R.J., Strid J. *et al*. (2010) Peanut sensitisation and allergy: influence of early life exposure to peanuts. *British Journal of Nutrition* **103**, 1278–1286.

Torheim L.E., Ferguson E.L., Penrose K. & Arimond M. (2010) Women in resource-poor settings are at risk of inadequate intakes of multiple micronutrients. *Journal of Nutrition* **140**, 2051S–2058S.

Tripp K., Perrine C.G., de Campos P., Knieriemen M., Hartz R., Ali F. *et al*. (2011) Formative research for the development of a market-based home fortification programme for young children in Niger. *Maternal and Child Nutrition* **7** (Suppl. 3), 82–95.

Victora C.G., Adair L., Fall C., Hallal P.C., Martorell R., Richter L. *et al*. (2008) Maternal and child undernutrition: consequences for adult health and human capital. *Lancet* **371**, 340–357.

Vitta B. & Dewey K.G. (2012) Identifying Micronutrient Gaps in the Diets of Breastfed 6–11-month-old infants in Bangladesh, Ethiopia, and Viet Nam using Linear Programming. Alive & Thrive: Washington, DC.

Whittaker P.G., Barrett J.F. & Lind T. (2001) The erythrocyte incorporation of absorbed non-haem iron in pregnant women. *British Journal of Nutrition* **86**, 323–329.

Woodhouse L.R., Abrams S.A., Chen Z., Griffin I.J., King J.C., Mendoza C. *et al*. (2008) Iron supplementation and changes in iron absorption and iron status during pregnancy and lactation. *FASEB Journal* **22**, 694.691.

World Bank (2006) *Repositioning Nutrition as Central to Development: A Strategy for Large Scale Action*. The World Bank: Washington, DC.

World Health Organization (1998) *Complementary Feeding of Young Children in Developing Countries: A Review of Current Scientific Knowledge*. World Health Organization: Geneva.

World Health Organization (2007) Conclusions and recommendations of the WHO consultation on prevention and control of iron deficiency in infants and young children in malaria-endemic areas. *Food and Nutrition Bulletin* **28**, S621–S627.

World Health Organization/Food and Agriculture Organization (2004) *Vitamin and Mineral Requirements in Human Nutrition*, 2nd edn. World Health Organization: Geneva.

World Health Organization/World Food Program/Standing Committee on Nutrition/United Nations Children's Fund (2007) Community-based management of severe malnutrition. A joint statement by the World Health Organization, the World Food Programme, the United Nations System Standing Committee on Nutrition, and the United Nations Children's Fund. World Health Organization: Geneva.

Yang Z. (2010) Are peanut allergies a concern for using peanut-based formulated foods in developing countries? *Food and Nutrition Bulletin* **31**, S147–S153.

Zhou S.J., Gibson R.A., Crowther C.A. & Makrides M. (2009) Should we lower the dose of iron when treating anaemia in pregnancy? A randomized dose-response trial. *European Journal of Clinical Nutrition* **63**, 183–190.