IRIS I: A FOODlet-based multiple-micronutrient intervention in 6- to 12-month-old infants at high risk of micronutrient malnutrition in four contrasting populations: Description of a multicenter field trial

International Research on Infant Supplementation (IRIS I) Study Group:
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Editors' Note

The overall plan for execution of IRIS originated in the 1999 workshop in Rio de Janeiro; that meeting produced an overall scope and approach to intervention later refined into a multicenter protocol. It took the work of a number of professionals around the world and analyses of biochemical samples and tabulated data to bring us a preliminary look at the experience and findings from the actual IRIS study to the plenary discussions at the Lima gathering. Only a short interval had elapsed between the conclusion of work in the field and laboratories and the convening of the workshop. The broad authorship of this paper recognizes the full array of participants in IRIS I activity. The results and findings presented by the teams in Lima were in a preliminary state of analysis, had not been subjected to combined across-site analysis, and have yet to be fully analyzed for publication. However, the plenary sessions provided an inventory of just how close (or how far) to the intended procedural mark were the specific individual trials in Peru, South Africa, Vietnam, and Indonesia. This paper presents this inventory and draws out the valuable lessons learned for the general practice of multinational common-protocol field investigation.

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Abstract

Infants in developing countries are at risk of concurrent micronutrient deficiencies, because the same causative factors may lead to deficiencies of different micronutrients. Inadequate dietary intake is considered one of the major causes of micronutrient deficiencies, especially among poor and underprivileged children in developing countries. Operational strategies and distribution systems are often duplicated when supplementation programs for single micronutrients are implemented at the same time. The International Research on Infant Supplementation (IRIS) trial was conducted in four distinct populations on three continents: Africa, Latin America, and Asia. The participating countries were South Africa, Peru, Vietnam, and Indonesia. The study had a randomized, double-blind, placebo-controlled design. Each country aimed to enroll at least 70 infants per intervention group (65 + 5 anticipated dropouts). The micronutrient vehicle was in the form of a "foodlet" (food-like tablet) manufactured as chewable tablets, which were easy to break and dissolve, and which had the same taste, color, and flavor for all countries.

Children were randomly assigned to one of four 6-month intervention groups: group 1 received a daily foodlet containing multiple micronutrients; group 2 received a daily placebo foodlet containing no micronutrients; group 3 received a weekly foodlet that contained multiple micronutrients (twice the dose of the daily foodlet) and placebo foodlets on the other days of the week; group 4 received a daily foodlet containing only 10 mg of elemental iron.

The IRIS Trial aimed to examine the prevalence of multi-micronutrient deficiencies in 6- to 12-month-old infants from rural populations, and to examine the efficacy of multi-micronutrient supplementation in infants from the different countries included in the study. This paper describes the general methodology of the IRIS trial and the operational differences among the country sites.

Key words: developing countries, foodlet, infants, multicenter trial, multi-micronutrient supplements, supplementation

Introduction

In the developing world, many vulnerable population groups suffer from multiple nutrient deficiencies. Of these deficiencies, iron, vitamin A, and iodine are the most commonly reported, although there may also be deficiencies of other vitamins and minerals. Infants are likely to have multiple, concurrent deficiencies, because the same causative factors may lead to a deficiency of different micronutrients. Cereal-based diets, rich in phytate and low in animal products, which predispose people to insufficient absorption of both iron and zinc, are commonly consumed in developing countries [1]. Micronutrient deficiencies are of global importance, as exemplified by several nutrition goals set in the early 1990s, including the virtual elimination of vitamin A and iodine deficiency, the reduction of iron-deficiency anemia in women by one-third, and the halving of severe and moderate undernutrition in children under 5 years of age by the year 2000. Although there have been considerable advances toward achieving these goals, a realignment of some goals adopted at the United Nations General Assembly Special Session on Children, May 2002, has been necessary due to challenges in research and implementation. The revised goals include reducing the percentage of children under the age of 2 with stunted growth by at least one-third, establishing sustained elimination of iodine deficiency disorders by 2005, and reducing the prevalence of anemia, including iron-deficiency anemia, in pregnant women and infants younger than 2 by at least one-third of the 2000 level by the year 2010.

Adequate intake of iron, vitamin A, zinc, and iodine is essential for growth and mental and motor development, and for the prevention of disease. Children with micronutrient deficiencies often present with linear growth retardation, higher morbidity and mortality, and reduced psychomotor development, preventing them from developing to their full potential [2, 3]. Exclusive breast-feeding during the first 6 months of life is considered sufficient to provide adequate nutrition for infants [4]. The transition to complementary foods in the process of weaning children to household diets, however, is associated with risks of nutrient deficiencies [5], due to the low density and/or poor bioavailability of certain nutrients in some household diets. It is well known that anemia is common in infants even in industrialized nations [6, 7], and that obtaining sufficient zinc from dietary sources is a challenge in most settings [5]. The consequence may be widespread prevalence of deficiencies of iron, zinc, and other assorted micronutrients from 7 months of age onward, especially in low-income societies [8, 9]. Providing for optimal nutrition status of infants born under the least favorable socioeconomic and climatic conditions would require exclusive breast-feeding during the first 6 months of life, with the possible application of routine dietary enrichment with selected micronutrients during the period of weaning transition [10].

It is theoretically possible that the currently recommended daily intakes of nutrients for infants, based on the US recommended dietary allowances (RDAs) (part of the *Dietary Reference Intakes*, or DRIs) [11], for instance, are not yet refined to genuine suitability. Or, they may indeed be correct for infants in the industrialized settings in which they are derived, but are not applicable to situations in which the majority of the populace is not "healthy," as defined in the disclaimers for application of the RDAs [11, 12]. The environmental stresses of extreme climate, parasites, poor sanitation, and recurrent infections may contribute to higher demands or a greater loss of nutrients; alternatively, the slower growth of infants may produce an imbalance between an abundant micronutrient offering and lowered total body demands. However, for the context of prophylaxis and support of micronutrient nutriture for an infant population, the dosages of published recommended levels would seem a reasonable and prudent point of departure [10].

The International Research on Infant Supplementation (IRIS) initiative arose in the context of the aforementioned public health questions [10]. It realized that before generalization of any supplementation of micronutrients at the programmatic level (even at the prudent dosages of approximately 1 RDA) could be justified, any issue regarding its efficacy and safety would have to be resolved. For the purposes of a robust initial evaluation, the IRIS group opted for a multicenter approach, in which the diverse ethnic and environmental context of three low-income regions—Latin America, Africa, and Asia—would be included, and the study populations would be subjected to a common intervention protocol, with the inclusion for reference of a no-treatment (placebo) control. The micronutrient vehicle would be an innovative feature, in the form of a "foodlet" (food-like tablet), conceived in consultation with international experts in Rio de Janeiro [10].

The primary format for supplementation administered essential micronutrients, dosed at infant RDA levels, every day for 6 months. Combining multiple micronutrients in a single delivery mechanism has been suggested as a cost-effective way to achieve multiple benefits [13, 14]. Some have questioned the effectiveness of nutrients combined within a supplement because of possible interactions of the nutrients or interference in their absorption [15, 16]. Given the specific recalcitrance of redressing iron nutrition problems at the public health level, a daily iron-only treatment group was added to identify any potential factors of nutrient-nutrient interactions related to iron in combination with other vitamins and minerals. Finally, because of data showing that intermittent (e.g., weekly) micronutrient supplementation could be less expensive and easier for participants to follow [17], a weekly (compared with daily) supplement was a final feature of the four-arm, randomized design.

Thus, a protocol was developed to explore the efficacy of multi-micronutrient supplementation in infants from micronutrient-deficient populations on three continents. After verification and inputs from the various participating countries, namely, Indonesia, Vietnam, Peru, and South Africa, the objectives of the study were as follows: (1) to examine the prevalence of multi-micronutrient deficiencies in infants from rural populations of these four selected countries; and (2) to assess the efficacy of multi-micronutrient supplementation in these infants on three parameters: the selected micronutrient status, anthropometric status, and morbidity status. This paper describes the general methodology of the IRIS trial and the operational differences between the country sites.

Populations, subjects, materials, and methods

Populations

Four geographically distinct populations from three continents participated in the study. The participating countries were South Africa, Peru, Vietnam, and Indonesia. Settings with a historical prevalence of anemia (defined as hemoglobin < 110 g/L) and vitamin A deficiency (< 0.7 μmol/L), ranging up to 30% of the preschool (under 5 years of age) population, were selected for an inquiry into the nutrition status of infants during their second semester of life.

In South Africa, the study was carried out in the geographic area of the Valley of a Thousand Hills, situated approximately 40 km northwest of the coastal city of Durban in the KwaZulu-Natal Province. This is a rural region in which families are scattered over a very large mountainous area. The community is predominantly Zulu. In Peru, the study was carried out in peri-urban communities of two cities of the department of Lambayeque (Lambayeque and Chiclayo), located approximately 600 km north of Lima, the capital of Peru. In Vietnam, the study was conducted in four communes (Phu Minh, Duc Hoa, Xuan Thu, and Phu Lo) in the rural district of Socson, 50 km North of Hanoi City. The Indonesian study was conducted in two subdistricts of Salam and Ngluwar, district of Magelang, province of central Java, Indonesia.

For ethical considerations the guidelines of the Council of International Organizations of Medical Sciences [18] were followed. Before the study, mothers were informed about the purpose and different phases of the study. The ethical review committees of each of the institutions approved the protocols and planned procedures and supervised the protection of autonomy and exclusion of undue risk for the participants. Parents or legal guardians of each participating child gave witnessed, informed (written) consent.

Selection of subjects

Within the populations, various randomization strategies were used to pre-select children for potential inclusion from the infant population of the given community. Eligibility for enrollment was determined by inclusion criteria that included being a stable resident of the catchment's area of interest, being of either sex, and having more than 180 and less than 365 days of age for the moment of initial measuring and sampling. Exclusion criteria for non-eligibility included failure to obtain signed informed consent, premature birth (< 37 weeks gestation), low birth weight (< 2500 g), severe wasting (< 3 Z-scores), anemia (hemoglobin < 80 g/L), and fever (> 39°C) on the day of blood sampling.

Study design and enrollment process

The study had a randomized, double-blind, placebo-controlled design. Children were randomly assigned to one of four intervention groups. Through the use of coding, the subjects' families as well as the investigators were blinded to the specific composition of the foodlets assigned. If a family had more than one child who was eligible to participate in the study, they were treated as separate cases, but were allocated to the same intervention group. Each country received a simple computer program to use in their randomization process. The sample size was calculated based on a power index of 2.86 and an anticipated dropout of about 7%. Thus, each country aimed to enroll at least 70 infants per intervention group (65 + 5 dropouts).

All subjects participated for 6 months. Group 1 received a daily foodlet containing multiple micronutrients. Group 2 received a daily placebo foodlet containing no micronutrients. Group 3 received a weekly foodlet containing multi-micronutrients at twice the dose of the daily foodlet and placebo foodlets on the other days of the week. Group 4 was given a daily foodlet containing 10 mg of elemental iron.

The RDA multi-micronutrient (MM) foodlet (daily dose) contained 1 RDA micronutrients for children aged 1 to 2 years, with the exception of zinc, which was the equivalent of 1 RDA for children aged 6 to 12 months. The weekly 2 RDA MM foodlet was equivalent to twice the daily dose. All foodlets had a piña colada flavor to make them acceptable to infants. The placebo foodlet contained no micronutrients. A week's supply was distributed in coded blister packs. Ingredients and dosing for the 1 RDA supplement are presented in table 1.

Roche Laboratories (New York, USA) was responsible for the elaboration of the final blend of the product, and a private laboratory in Peru (Hersil SA, Peru) was responsible for the production and quality control of the supplements. Foodlets were manufactured as chewable tablets, which were easy to break and dissolve, and which had the same taste, color, and flavor for all countries. Three of the groups (placebo, iron, and daily multi-micronutrients) had blisters with seven tablets and within each group all tablets had the same composition; the fourth group (weekly multi-micronutrients) had blisters that included six placebo tablets and one 2 RDA tablet placed always in the same position within the blister arrangement. For all intervention groups, the first foodlet used from a blister was from this position. Before the final production, a double-blind acceptability trial was carried out in the study area of Peru with the participation of 50 infants and their mothers. Results showed that there was good acceptability of the four presentations. The other countries also found the foodlets very acceptable by their respective target populations. Coded blisters were distributed to all participating countries. Codes were kept by UNICEF, New York, and only broken at the end of the study, prior to statistical analyses.

Consumption of foodlets

In Indonesia and Peru, the foodlets were administered to the babies at home on a daily basis for 7 days. On six of the days, a trained field worker/assistant gave the foodlets directly to the babies and on the seventh day the mother gave the supplement to the infant. Compliance with the administration of the first six supplements to the infants was recorded directly, and that of the seventh during the next day's visit. In Vietnam, the foodlets were given daily to the infants under close supervision of the commune health workers, who recorded compliance. In South Africa, all blisters and working materials were color-coded to facilitate and simplify the identification of groups. Mothers were provided with one month's foodlet supply in a color-coded container, because the study was done in a rural, scattered area and it was impossible for community health workers to visit all households on a daily basis. The mothers and/or caregivers were given demonstrations on how to how to crumble and mix the foodlet with porridge. Mothers were trained to mix foodlets with a small quantity of porridge (maize meal predominantly), as it was important that the child should eat the entire portion mixed. Compliance was monitored during weekly visits by the community health workers by means of a short questionnaire and observation (the number of foodlets removed from the previous week's blister was counted and recorded).

TABLE 1. Composition of 1 RDA multi-micronutrient foodlet.

Ingredients	Amount	Product form	% overage	mg per foodlet
Vitamin A	375 μg RE (1250 IU)	A acetate 325 CWS/F	35	5.2
Vitamin D	5 μg	D 100 CWS	35	2.7
Vitamin E	6 mg α-TE	E 50 CWS/F	10	13.2
Vitamin K	10 μg	0.5% Trit	50	3
Vitamin C	35 mg	Fine C powder	5	36.75
Vitamin B_1	0.5 mg	Rocoat 33 1/3%	10	1.65
Vitamin B_2	0.5 mg	Rocoat 33 1/3%	10	1.65
Vitamin B_6	0.5 mg	Rocoat 33 1/3%	7.5	1.61
Vitamin B_{12}	0.9 μg	0.1% WS	30	1.17
Niacin	6 mg	Niacinamide	5	6.3
Folate	150 μg	10% triturated	25	1.88
Iron	10 mg	Ferrous fumarate (32.87% Fe)		30.42
Zinc	5 mg	Zinc gluconate (14.35% Zn)		34.84
Copper	0.6 mg	Cupric gluconate (14% Cu)		4.3
Iodine	59 μg	1% potassium iodide trit		5
Milk solids				1,100
DiPac		Piña colada		600
6X Sugar		Calcium carbonate 90 A		600
Fructose				240
Flavor				52.4
Citric acid				22.4
Calcium carbonate				53
Magnesium stearate				13.5
		Supplement weight (mg)		2,830.97

Questionnaires

All information was collected in a closed questionnaire according to the recommendations of Gross et al. [19] (http://www.nutrisurvey.de). The questionnaire comprised four different components, namely, (1) the baseline or household questionnaire, which was only used at the beginning of the trials; (2) the monthly weight and height questionnaire, which also included the date of measurement to calculate with the age of the child the Z-scores; (3) the weekly health visit form; and (4) the daily health and infant feeding information questionnaire. All daily information obtained was converted to weekly information.

Anthropometry and assessment of growth

Infants' anthropometric measurements were measured on a monthly basis. Length was measured with the subject supine, using an infantometer measuring board (Ahrtag, London, UK), and recorded to the nearest 0.1 cm. Weight was determined with minimal clothing on electronic weighing scales (SECA, Hamburg, Germany). In South Africa, weight was measured to the nearest 50 grams on a load-cell-operated digital scale (UC-300 Precision Health Scale). All anthropometric measurements were taken by the same person in South Africa to minimize individual variation; similarly, in Vietnam, the same two people measured height and weight separately.

Blood collection and sample processing and handling

Blood was obtained from the vein with 2 ml–vacuette heparin tubes with butterfly luer-lock adapters (Greiner, Essen, Germany). The puncture site was cleaned carefully with cotton wool and 70% alcohol. After collection, tubes were immediately put in a cooled styropore box with coolpacks and centrifuged within 6 hours at 5000 g for 5 minutes. Plasma and erythrocytes were stored at –70°C until transportation on dry ice to Germany where they were analyzed in the Micronutrient Laboratory of the Institute of Biological Chemistry and Nutrition at the University of Hohenheim (the exceptions were hemoglobin and urinary iodine, which were measured at each site). All materials used for blood taking and storage were from the same batch and provided by the biochemical laboratory in Germany.

Hematologic assessment

Hemoglobin concentrations were determined with the cyanomethemoglobin method [20] by using a kit from Sigma (Sigma, St. Louis, USA); 20 µl whole blood were mixed with 5 ml Drabkins solution and measured by a standard photometer and quantified with a Hb standard. All populations were located within 500 meters of sea level, such that no altitude adjustment was needed.

Biomarkers of mineral nutriture

Serum ferritin was measured by a standard sandwich ELISA procedure from the provider of the antibodies (DAKO, Hamburg, Germany). Plasma zinc and copper concentrations were analyzed by flame atomic absorption spectrophotometry according to the description of the manufacturer (Perkin Elmer, Frankfurt, Germany). Urinary iodine concentrations were assessed based on alkaline digesting using the Sandell-Kolthoff reaction [21].

Biomarkers of vitamin nutriture

Retinol, α-tocopherol, and β-carotene were analyzed according to Erhardt et al. [22]. The plasma proteins were denatured by mixing 30 µl plasma with 150 µl ethanol/n-butanol (1:1 v/v, containing 5 mg BHT/ml and 4 µmol/l Tocol as internal standard). The mixture was then centrifuged for 5 minutes at 12,000 g and 30 µl of the supernatant were analyzed by using reversed phase high-performance liquid chromatography (HPLC). For homocysteine as an indicator of folic acid status, the HPLC method of Pfeiffer et al. [23] was used. Homocysteine status is also influenced by vitamin B_6 and vitamin B_{12}. For the riboflavin status, the method of Bayoumi and Rosalki [24] was used by calculating the activation coefficient of the erythrocyte glutathione reductase with and without added riboflavin.

Biomarkers of activation of the acute-phase response

Infectious status was measured by C-reactive protein (CRP) for short-term effects and by α-1-acid glycoprotein (AGP) for long-term effects. For these measurements, a Sandwich ELISA was used according to the description of the manufacturer of the antibodies (DAKO, Hamburg, Germany). Elevation for CRP was considered to be a value of > 12 mg/L, whereas abnormally high AGP was a concentration of > 1 g/L. The status of an abnormally increased acute-phase-response marker was taken into consideration for the diagnostic assessment and interpretation of retinol, zinc, and iron status markers. Table 2 presents a summary of anthropometric measurements and all biochemical analyses done on blood samples, as well as the time of measurement.

Data processing and statistical analysis

All collected data were entered into a spreadsheet using SPSS. The Medical Research Council, South Africa, will carry out the statistical evaluation of the data collected at all sites.

TABLE 2. Anthropometric and biochemical variable indicator matrix and time of measurement.

Variable	Indicators	Time of Measurement
Anthropometry	Weight	Monthly
	Height	Monthly
Morbidity	Diarrhea, acute respiratory infection, fever	Daily*
Iron status	Hemoglobin	Begin/end
	Plasma ferritin	Begin/end
Vitamin A status	Plasma retinol	Begin/end
	Plasma β-carotene	Begin/end
Vitamin B status	Plasma homocysteine	Begin/end
	Plasma riboflavin	Begin/end
Vitamin E status	Plasma α-tocopherol	Begin/end
Zinc status	Plasma zinc	Begin/end
Copper status	Plasma copper	Begin/end
Iodine status	Urinary iodine excretion	Begin/end
	Iodine content in the household salt	At least begin/end
Acute phase proteins	C-reactive protein	Begin/end
	α-1-acid glycoprotein	Begin/end

* On a weekly basis in South Africa.

Discussion

At the International Workshop on Multi-Micronutrient Deficiency Control in the Life Cycle in Lima, Peru (May 30–June 1, 2001), it was indicated that the question of efficacy and safety of multiple micronutrient supplementation must be addressed before programs can be devised and implemented [25]. The IRIS trial should provide concrete evidence of efficacy of multimicronutrient supplementation in infants with a diverse background from three continents, and some evidence of safety. The "foodlet" concept was conceived at the Rio de Janeiro meeting to have multiple attributes [10]. Firstly, the intention was to move nutrient supplementation out of the medicinal form and dominant health-sector-control arena and into a dietary and food context. This accepts that matrix factors might reduce the net bioavailability as compared with that in a capsule or elixir, but this would be an acceptable tradeoff if compliance were enhanced proportionately. The foodlet was conceived of to be a crumbly, cookie-like item, a "tabletted snack" that could be either sucked to dissolution by edentulous infants, or gnawed or chewed by babies with teeth, or crumbled and sprinkled onto a porridge or pap, or mixed into gruels or beverages. Each country was allowed to administer the foodlets to infants by means of an acceptable practice for that specific community. Inter-mealtime consumption with a high compliance was managed in three of the sites. In Vietnam, for instance, the foodlet was dissolved in a little water in a cup and given with a spoon to the infant. In South Africa, the foodlet was crushed and mixed into the traditional maize gruel as the introduction of weaning food that usually begins during early infancy there. Nevertheless, compliance was good and estimated to be well above 90%.

The intervention period was 6 months for all participating countries, except for Indonesia, where the period fell one week short due to a big Moslem festival of *Iedul Fitri*, which was celebrated by the majority of study subjects. Research activity in the community during that period was not recommended. Further, all countries followed the protocol closely, taking into account their own unique situations and circumstances. The only deviation occurred in South Africa, where infants did not take the supplement under close supervision of study workers due to the rural geographic area where the study subjects were scattered. Therefore all compliance and morbidity data were only collected on a weekly basis there. Thus, the South African leg of the study was not executed as a typical efficacy trial in which supplements are taken under close supervision, but more in a deep "rural efficacy" style where mothers and caregivers are trained and empowered to play the supervision role. The South African study can therefore be seen as a hybrid between an efficacy and an effectiveness trial that should provide specific information on how to do efficacy trials in deep rural areas under remote conditions, where the most vulnerable populations for micronutrient deficiency often reside.

A strong point of the IRIS trial was that the biochemical analyses were done centrally, at one laboratory, which also provided all material for blood sampling. The laboratory uses micromethods (< 30 µl for each measurement) to reduce the necessary volume of blood as much as possible. Further, it also measures a wide spectrum of indicators including metabolic variables, such as homocysteine.

Conclusion

In conclusion, the outcome of the IRIS I trial should yield unique information on how effectively combined multiple micronutrients can combat micronutrient deficiencies during the second semester of infancy, taking into account the possible interactions and interference in their absorption. This will all be gauged against the consequences that multi-micronutrient supplementation might have on anthropometric and morbidity indicators, using no supplementation or iron-only supplementation as a reference.

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