Title of the Study: Effect of daily consumption of a micronutrient-fortified, energy-dense lipid-based nutrient supplement (LNS) on breast milk intake in Malawian infants

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Background and design
This study is designed as a sub-study within a larger randomized controlled trial (called "iLiNS-DOSE") that we are currently conducting in rural Malawi to evaluate the effect of daily consumption of a micronutrient-fortified, energy dense, lipid-based nutrient supplement (LNS) on growth and development in infants.

The World Health Organization recommends that infants receive breast milk exclusively until they reach 6 months of age. However, infants in rural Malawi typically receive maize porridge at 3-4 mo of age, and begin to experience growth faltering by about 4 mo of age. By 18 mo of age stunting and underweight are common and 30-50% of all Malawian children <5 y of age are stunted or underweight (UNICEF, 2006). Provision of LNS, in the form of a paste that can be added directly to complementary foods prepared in the home beginning at 6 mo of age, may help prevent growth faltering in early childhood. Preliminary results from Malawi and Ghana suggest that daily consumption of 20-50 g of LNS for 6-12 months increases linear growth and markedly reduces the incidence of severe stunting before the age of 18 months.

The iLiNS-DOSE trial aims to identify the lowest daily dose of LNS that promotes growth in infants when given between the ages of 6 and 18 months (see full trial protocol at ClinicalTrials.gov, NCT00945698). Specifically, we will assess the growth-promoting effects of LNS given daily to infants at a dose of 10 g, 20 g or 40 g, from 6 to 18 months of age; a comparison group will not receive any study supplement until after 18 months (when they will receive fortified maize-soy porridge). The daily doses of 10 g, 20 g or 40 g of LNS will provide 56 kcal/d, 118 kcal/d and 243 kcal/d, respectively. Mothers will be encouraged to breastfeed their infants on demand during the 12-month intervention. It is possible that children may reduce their intake of breast milk when they are provided with energy-dense LNS-containing complementary foods. Because a reduction in breast milk intake could have a negative impact on infant health, we are assessing breast milk intake in infants participating in the larger intervention trial, at 9-12 months of age.

Specific objective
To estimate breast milk intake in a sub-sample of Malawian infants participating in the LNS intervention trial at 9-12 months of age using the dose-to-mother deuterium oxide dilution technique.

Hypothesis
Mean breast milk intake (g/d) will not differ by group in the sub-sample of infants participating in the larger LNS trial at 9-12 months of age.

Study procedures and research methods

Study procedures: Initially, mothers are weighed to the nearest 0.1 kg using an electronic scale, and measured to the nearest 0.1 cm using a stadiometer. Infants are weighed to the nearest 0.01 kg using an electronic scale; and measured to the nearest 0.1 cm using a length board. After completing the anthropometric measurements, saliva samples are obtained from mothers and infants. Saliva samples are collected from mothers by carefully placing a small piece of cotton wool (cotton ball) in the mother's mouth and asking her to keep it in her mouth for about 1-2 minutes until it is soaked with saliva. When the cotton wool is fully soaked, the mother is asked to position the cotton wool in the front of her mouth and transfer it directly to the empty barrel of a 20 mL disposable syringe (to prevent contamination of the cotton). The saliva is squeezed out of the soaked cotton wool into a small screw-cap vial. The procedure is repeated using a new piece of cotton wool, if necessary, until 2-4 mL (~1/2 to 1 teaspoon) of saliva is collected. Saliva samples are collected from infants by carefully placing a cotton wool swab into the infant's mouth for 1-2 minutes until the cotton wool is soaked with saliva. The swab is removed from the infant's mouth and the cotton is separated from the swab and placed into the empty barrel of a 20 mL disposable syringe. The saliva is squeezed out of the soaked cotton wool into a small screw-cap vial. The procedure is repeated until ~ 2 mL (½ teaspoon) of saliva is collected from the baby. The vials are tightly capped and stored at -20^{o}C until analyzed for deuterium enrichment.

After collection of the baseline saliva samples, mothers are asked to drink 30 g (~ 1 ounce) of deuterium oxide from a small polypropylene bottle through a straw. The empty bottle is filled with ordinary drinking water (50 mL) and the mother is asked to drink the water to ensure that all of the deuterium oxide is consumed. Saliva samples are collected from mothers and infants again at 1, 2, 3, 4 and 13, and 14 days after dosing, using the same procedures described above.

Because intake of other foods and illness could potentially affect breast milk intake, we are also assessing infant dietary intake by conducting two repeat 24-hr recalls with the infant's primary caretaker, and we are assessing infant and maternal morbidity weekly by interview.

Selection of subjects: Infants are randomly selected concurrently from each of the four study groups in the larger LNS intervention trial at the time of enrollment (6 mo of age); however procedures for the sub-study do not begin until infants are 9-12 months of age. Enrollment will be on-going during a one-year period. A total of 400 mother-baby pairs will be selected for the sub-study. Informed consent is obtained from mothers who choose to participate with their infants. The study protocol has been approved by the Institutional Review Boards of the College of Medicine, University of Malawi and the University of California, Davis.

Measurement of deuterium enrichment of saliva samples: The deuterium enrichment of saliva samples is measured using Fournier Transform Infra-red Spectroscopy (FTIR) (Jennings, 1999). Briefly, saliva samples are thawed at room temperature and centrifuged at 1000g for 10 minutes. The FTIR cell is filled with saliva and absorbance is measured between 2300 – 2900 nm. Deuterium enrichment of the samples is calculated using a standard curve that is developed with standards of known deuterium enrichment.

Estimation of average daily breast milk intake: Average daily breast milk intake will be calculated from deuterium enrichment data from mother-infant pairs using a two compartment, steady-state model, as previously described (Coward, 1982).

Dietary intake: Dietary intake of infants is assessed by interviewing the infant's primary caretaker using the 24-hr recall method.

Morbidity: Maternal and infant morbidity is assessed weekly by asking the mother to recall whether she or her infant experienced selected symptoms of illness during the previous week using a questionnaire.

Sample size: The sample size was based on a minimal detectable difference in breast milk intake that represents less than 10% of total energy needs at 9-11 months of age. A difference of 86 g/d in milk intake represents approximately 55-60 kcal, which is 8-9% of total energy needs of 686 kcal/day at 9-11 months of age (Dewey & Brown, 2003). Assuming average milk intake at 9-11 months of age is 616 g/d ± 172 g/d (WHO, 1998), a sample size of 89 mother-infant pairs per group is needed to detect a difference in milk intake of 86 g/d by group, (α=0.05, β=0.80, effect size=0.5). The sample size was increased to 100 per group (total of 400) to account for attrition.

Data analysis: Mean breast milk intake will be compared by study group using analysis of covariance.

References:

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