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B. IRON STATUS OF NICARAGUAN CHILDREN BY SERUM FERRITIN ASSESSMENT AT BASELINE AND FOLLOWING INTERVENTION WITH VITAMIN A-FORTIFIED SUGAR

This part of the project was added on to the initial proposal to the Micronutrient Initiative as the opportunity to assess iron status became available in the Nicaragua Sentinel Survey. The hypothesis was that intervention with vitamin A- fortified sugar would be associated with an improvement in iron status (Lynch 1997).

Objective.

Assessment of iron status based on serum ferritin in Nicaraguan children at baseline and during follow-up after intervention with vitamin A- fortified sugar.

Methods.

DSS samples prepared by precise aliquot of 20 μ L serum on Whatman No. 1 filter paper (Whatman Inc, Clifton, NJ) were sent to Dr. Ahluwalia's laboratory by courier within a week of collection in the Nicaraguan Sentinel Survey, at baseline and in the middle of the survey (time point 3) and at the end of the survey (time point 5). Liquid serum samples were stored frozen and sent on dry ice from each of these rounds for the determination of the positive acute phase reactant protein AGP, by radial immunodiffusion using a commercial kit (Kent Laboratories, Richmond, WA). Spot ferritin assay with modifications as outlined in the first part of this report, was carried out on DSS samples at 2 wk from date of collection. Pooled serum controls were included to account for batch-to-batch variation (CV: 4%).

Statistical Analyses.

Logarithmically transformed data for serum ferritin were used for statistical analyses as log-transformed distribution were consistent with normality. Children were first classified as having inflammation based on AGP \geq 110 mg/L (Filteau et al. 1993). Then they were

characterized as iron-deficient (DSS ferritin $< 16 \mu\text{g/L}$) or iron-sufficient (DSS ferritin $\geq 16 \mu\text{g/L}$). Because of the lack of complete information at the present time on potential confounders such as baseline vitamin A status, gender, age, hemoglobin, and sugar retinol intake regression analyses and repeated measures analyses to examine longitudinal effects of intervention could not be undertaken. Thus, simple univariate examination of the data were possible at the present time.

Results and discussion

The cross-sectional analysis of the distribution of children by presence of inflammation is shown in Table 4. A trend of reduced inflammation in children with use of vitamin A-fortified sugar was noted (Table 4). This is in agreement with the vast literature supporting the role of vitamin A in improving immune response and reducing infection, inflammation, and morbidity (Bahl et al. 1995; Rahman et al. 1997; Villamor and Fawzi 2000)

Table 4: Cross-sectional distribution of Nicaraguan children by presence or absence of inflammation

Inflammation	Time 1	Time 3	Time 5
Yes (AGP $\geq 110 \text{ mg/L}$)	22 (17%)	16 (12 %)	11 (8 %)
No (AGP $< 110 \text{ mg/L}$)	108 (83%)	114 (88 %)	118 (91 %)
TOTAL N	130	130	130

Children with or without inflammation at each time point were further subclassified as iron-deficient or iron-sufficient based on serum ferritin values obtained by spot assay on DSS samples using a cut-off of $16 \mu\text{g/L}$ for children without infection (Cook et al. 1986) and $50 \mu\text{g/L}$ for children with infection (Ahluwalia et al. 2001). The distribution of children by iron status based on serum ferritin determination at each time point shows no significant trends with

vitamin A- fortified sugar intervention (Table 5).

Table 5. Cross-sectional analysis for the distribution of Nicaraguan children by iron status and inflammation with vitamin A- fortified sugar intervention

Inflammation	Iron Status	Time 1	Time 3	Time 5
Yes (AGP \geq 110)	Iron-sufficient	0 (0 %)	2 (2 %)	1 (1 %)
	Iron-deficient	22 (17 %)	14 (11 %)	11 (8 %)
No (AGP < 110)	Iron-sufficient	75 (58 %)	82 (63 %)	74 (57 %)
	Iron-deficient	33 (25 %)	32 (25 %)	44 (34 %)

Note: Iron deficiency is defined as serum ferritin < 16 $\mu\text{g/L}$ for children without infection and as serum ferritin < 50 $\mu\text{g/L}$ for children with infection.

The following table shows mean ferritin values for the children who were classified as iron-deficient or iron-sufficient in Table 5. This table also supports the findings from Table 5 that iron status did not change, at least when examined from a cross-sectional view point, with introduction and use of vitamin A- fortified sugar. It is imperative to compute how much additional vitamin A was indeed consumed per child and also account for other confounders in order to do longitudinal over time analyses in the future.

Table 6. Serum ferritin ($\mu\text{g/L}$) in Nicaraguan children with vitamin A- fortified sugar intervention: Results from cross-sectional analyses

Inflammation	Iron Status	Time 1	Time 3	Time 5
Yes (AGP \geq 110)	Iron-sufficient	N/a	59.7 (51.9 - 68.7)	53.5 (N/a)
	Iron-deficient	29.0 (20.5 - 41.3)	30.0 (17.4 - 51.4)	24.8 (15.6 - 39.3)
No (AGP < 110)	Iron-sufficient	25.8 (18.5 - 35.9)	26.0 (18.7 - 36.2)	25.5 (18.7 - 34.8)
	Iron-deficient	11.9 (8.8 - 16.1)	11.2 (8.2 - 15.3)	9.6 (6.5 - 14.2)

Note: Values represent geometric mean and values in parenthesis represent geometric mean \pm 1 SD.

Summary

A cross-sectional analysis of data on serum ferritin at 3 time points in the Nicaraguan Sentinel Study suggests that no change in the frequency distribution of children as iron-deficient or iron-sufficient occurred with the introduction of vitamin A- fortified sugar. However, it is important to note that longitudinal analyses are critical to examine the effect of such an intervention on iron status and these analyses would require coordination of various teams involved. Ideally, a team which is familiar with the design and the data collected could take leadership in these analyses. My suggestion would be that Dr. Bulux would be an ideal candidate for this role - if he has the time and is willing to take this commitment for compensation of his effort. Dr. Bulux is familiar with the design of the survey and the data collected, has trained Nicaraguan personnel involved in the survey to collect DSS samples, and is fluent in SAS and data analysis. I would also suggest that other investigators including myself, Dr. Neal Craft, Dr. Noel Solomons, Dr. Charles Wallace, and Dr. Erick Boy as well as other members working on the vitamin A part of the project from the Micronutrient

Initiative work together (via netmeetings and in individual meetings at Experimental Biology for instance, or in a meeting organized for these key individuals by the Micronutrient Initiative in a mutually convenient venue) in discussing these analyses and providing timely input for completion of these important data analyses and dissemination of these potentially significant results to the global community.

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