

Feature:

CeSSIAM celebrates 25 years of nutrition research

The Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) in Guatemala was founded on July 1, 1985, to assert the need for academic freedom in the pursuit of public health research. It allowed the researchers to move forward, rather than looking backward, in the development of a research agenda. Throughout the 25-year history of CeSSIAM, the study of micronutrients has been central to its scientific fabric, enabling progress in understanding the health implications of vitamin A, vitamin D, riboflavin, zinc, iron and iodine.

Vitamin A

The first study from CeSSIAM to be published in an international journal compared the dietary intakes of vitamin A as assessed in the same individuals by 24-hour recall or food frequency measurement [1]. It seemed that research on vitamin A was the thing to do in those days. A few years earlier, mandatory fortification of table sugar with vitamin A in Guatemala, by a process developed by Guillermo Arroyave at the Institute of Nutrition of Central America and Panama (INCAP), had failed because of resistance from the sugar industry.

Our patron organization, the Guatemalan National Committee for the Blind and Deaf, understood “nutritional blindness” in its relationship to hypovitaminosis A, and had been pivotal in the earlier movement to introduce mandatory sugar fortification. So, after Alfred Sommer and colleagues at the Johns Hopkins School of Hygiene and Public Health (JHSHPH) demonstrated a causal relationship between marginal vitamin A status and child mortality in Indonesia [2], two of the founding coordinators of CeSSIAM, the late Oscar Pineda and Fernando Beltranena, took up the challenge to document the magnitude of the vitamin A problem, and reassert public interest in universal sugar fortification. The breakthrough came when JHSHPH, in collaboration with CeSSIAM, established the conjunctival impression cytology method as a functional assessment of hypovitaminosis A [3]. This led civil society and the medical authorities to persuade the sugar industry to reinstate sugar fortification in 1987, and continue through all sugarcane harvests to the present day.

Extending CeSSIAM’s involvement with the assessment of vitamin A status, Jesus Bulux, working with Neal Craft (Craft Technologies, North Carolina, USA), demonstrated the validity of the dried blood spot approach (in which a drop of capillary blood is collected on filter paper) for accurate assessment



CeSSIAM 25th anniversary exhibit booth at the national Science Week 2010 celebration with staff members

of circulating retinol [4]. In a natural collaboration between CeSSIAM and the Human Nutrition Research Center on Aging (HNRC-A) at Tufts University in Boston (both organizations do research on aging) we examined the effect of advancing age on assessment of vitamin A status. After finding a poor within-individual reproducibility of the relative dose response in older individuals [5], we applied the deuterated-retinol-dilution technique (a stable-isotope label test) and found generally adequate liver stores [6]. Later, we showed that an abbreviated 3-day equilibration period was sufficient to provide a valid estimate of body stores of the vitamin [7].

CeSSIAM has also investigated the bioconversion of provitamin-A-carotenes to active vitamin A. In collaboration with Steve Schwartz, then at North Carolina State University, we produced an extensive

Collecting data in a CeSSIAM field study



update on the carotene composition of Guatemalan plants. From this experience, we developed a variety of sweet-potato flakes as a prototype of a potentially high-vitamin-A weaning cereal. Two experiments tempered our enthusiasm, however. We found that feeding carrots to schoolchildren, and distributing carrots, sweet potatoes and green herbs to rural households increased circulating concentrations of beta-carotene, but did not influence circulating retinol levels [8, 9]. At that time, it was assumed that six units of dietary beta-carotene or twelve units of other provitamin A carotenoids were converted to one unit of vitamin A in the body. Jesus Bulux and I challenged the validity of this convention. In a 1993 article in *Nutrition Reviews*, we reasoned from a series of angles that these efficiencies were overly optimistic [10]. This was shortly before Saskia de Pee and colleagues published a study in *The Lancet* that provided empirical evidence for a lower bioconversion efficiency of plant sources of provitamin A carotenoids [11]. As a consequence of these challenges, the Food and Nutrition Board of the US Institute of Medicine (IOM) set the equivalency factors for the bioconversion of dietary provitamin A carotenoids in the 2001 Dietary Reference Intakes (DRIs) at twelve and twenty four. Most recently, we have rejoined this aspect of inquiry with a field survey into the genetic variants of the enzyme beta-carotene monooxygenase-1. In collaboration with the laboratory of Georg Lietz in Newcastle, UK, we extracted DNA from the saliva of indigenous Guatemalans, and determined that the more efficient type of enzyme was common, consistent with the notion that evolutionary pressures from a high-plant, agrarian diet favored more effective extraction of vitamin A from provitamin-A-carotenoids.

Riboflavin

CeSSIAM played a major role in assisting the IOM in its mission to extend and differentiate age-specific nutrient recommendations for riboflavin. The HNRC-A in Boston had the mandate to fill the outstanding gaps in the estimation of recommendations beyond age 51 years to needs for those 70 years and older within the context of the DRIs. William Boisvert performed his doctoral dissertation, assisted in Guatemala by Ivan Mendoza, in a metabolic study among free-living elderly men and women in which the graded addition of riboflavin to the diet provided an estimation of the intake needed to maintain adequate status [12]. These data formed the basis for the extension of specific riboflavin intakes for the elderly in the 1998 IOM publication of DRIs for the B vitamins.

In the course of this experience, we were able to document the existence of riboflavin deficiency in Guatemalan children, its relationship to dairying and non-dairying areas across the country, and the parallel association of riboflavin and vitamin B12 status in the same individuals [13, 14]. In addition, in the metabolic studies of the elderly, we discovered that the higher



carbohydrate intakes in a day, the easier it was to extract and retain riboflavin from the diet [12].

Vitamin D

Vitamin D and calcium are most often discussed as a tandem in human nutrition. The maize tortilla, staple food of the traditional Guatemalan diet, is prepared and cooked with lime (calcium oxide), providing the nation with a very high calcium intake. Until last year, however, nothing was known about vitamin D nutrition in this country other than from dietary surveys at CeSSIAM and elsewhere showing meager consumption of foods and beverages rich in the vitamin. In collaboration with the Osteoporosis Center at Creighton University in Nebraska, USA, a team of CeSSIAM researchers evaluated the circulating 25-hydroxy-vitamin D concentrations in 115 elderly Mayan (indigenous) residents of the western highlands province of Quetzaltenango. Despite high altitude and outdoor activities, low or deficient vitamin D status was found in the vast majority of participants, with women having significantly lower blood vitamin levels than men. We suspect that the dominant factors for this are exclusion of sunlight to the skin due to the design of traditional Mayan dress, pigmentation and aging of the skin, and low dietary vitamin D intake.

Zinc

CeSSIAM has participated in two international collaborations investigating zinc nutrition. The first of these involved the laboratory of Rosalind Gibson at the University of Guelph, Canada. It set the stage for the zinc experience at the center with a master thesis project by Sian Fitzgerald, in which she documented a high zinc-to-phytate ratio in the habitual diet of periurban pregnant women [15]. Kelly Cavan from the same department led zinc studies in urban schoolchildren. Using a large battery of potential outcome variables, she found some cross-sectional associations

Noel Solomons and Odilia Bermúdez, Associate Professor at Tufts University School of Medicine, Boston, present the results of the vitamin D study in Guatemala

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between circulating and hair levels of zinc and function [16]. A randomized controlled field trial of zinc supplementation, however, failed to produce major improvements in any of the chemical or functional variables [17]. Manuel Ruz of Chile undertook some of his doctoral study time around this project to refine his comparative investigation of laboratory indicators of zinc status.

Michael Hambidge (University of Colorado Health Science Center) was the senior collaborator in our second adventure in zinc research. With funding from the Thrasher Foundation, we established metabolic studies in free-living rural children, and measured zinc absorption in meals containing a hybrid maize variety with reduced phytic acid, using stable isotopes of zinc [18]. This turned out to be a prelude to a multi-institutional collaboration as part of the Global Network for Women's and Children's Health of the National Institute for Child Health and Human Development of the US National Institutes of Health. In this study, the effect of both lowering the content of the phytic acid in maize and providing a daily supplement of 5 mg zinc was examined in relation to the growth and illness experience of infants from 6 to 12 months of life [19].

More recently, we have examined the intestinal uptake of zinc from tablets used widely in programs for oral rehydration, as well as growth or disease resistances as outcome variables. Because circulating zinc concentrations failed to rise appropriately in the subjects given zinc rather than placebo in field studies (including our own), we used a simple plasma zinc response test to determine the relative bioavailability of the zinc tablet compared to powdered zinc sulfate. In this study with healthy adult males, the tablet delivered only half as much zinc as the powder. This lesser uptake is probably unimportant for the treatment of acute diarrhea, as the site of action is likely within the intestinal lumen; for systemic use, however, a more absorbable zinc formulation might be indicated.

Iron

Iron is not only an essential nutrient; it is also a powerful oxidant, and therefore a two-edged sword in human nutrition. Two of our early field trials on iron showed that preschool children had a prevalence of anemia greater than 20% [20], whereas schoolchildren across the country had less than a 12% rate [21]. Later, our research agenda added the notion of how to improve iron status. In a randomized controlled trial, we showed that adding hemin, the iron-containing entity in bovine blood, to refried black beans improved the hematological status of anemic preschool children [22].

In a series of iron intervention trials, we also examined how iron status, as indicated by ferritin, affected biomarkers of inflammation. Alpha-1 antichymotrypsin, but not other acute-phase response proteins or C-reactive protein (CRP) increased their circulating concentrations during daily supplementation of 20 mg iron to schoolchildren [23]. In a similar population we



CeSSIAM staff, July 2010

established, in collaboration with Laura Murray-Kolb and the late John Beard, that immunostimulation, which might confound interpretation of ferritin as an index of iron status, was low [24]. More importantly, we identified different individuals expressing primarily CRP or alpha-1 globulin as the marker of an active inflammatory state.

After the discovery of hepcidin, we collaborated with research groups in Nijmegen, the Netherlands, and Innsbruck, Austria, equipped with assays for active hepcidin and prohepcidin, to assess the relationship of iron status and iron administration to the two hepcidin forms in blood and urine. High iron stores suppressed hepcidin. The adverse consequences of oral supplementation with 12.5 mg iron to infants and toddlers, implicated "free iron" (non-transferrin-bound iron or NTBI) after oral dosing as a contributing factor. CeSSIAM studies have simulated the dosing of oral iron to children with proportionally increased iron doses in adult men. We found that ferrous sulfate results in a burst of NTBI, whereas two other bioavailable iron compounds, NaFeEDTA and iron polymaltose,

Caitlin Crowley measuring hemoglobin non-invasively



had a markedly subdued post-supplementation rise in levels of NTBI.

Monica Orozco of the CeSSIAM staff examined the effects of iron supplements on the colon and its antioxidant buffer capacity in her doctoral dissertation research at the University of Manitoba, Canada. She developed a modified assay for free-radical production in human feces, and showed that oral supplementation with iron eroded the capacity of the fecal material to buffer free-radical formation. She went on to show that concomitant supplementation with extracts of palm oil containing high doses of carotenes mitigated this reduction [25].

Iodine

The idea of using the more stable and robust iodate salts instead of iodide for fortification of salt in humid, tropical countries originated in Guatemala, which has a large number of small-scale salt producers. CeSSIAM has analyzed commercial salt packages from rural areas and documented a wide heterogeneity of fortification. Nicole Umemoto showed that education of schoolchildren could lead to the purchase of the superior brands in their homes [26].

CeSSIAM collaborated with the Centers for Disease Control and Prevention of the USA to standardize an ultrasound-based instrument for measuring the volume of the thyroid gland as a means of assessing goiter prevalence [27]. Finally, in research for a doctoral dissertation, Jesus Bulux showed that oral doses of iodine in oil given to lactating mothers were capable of enriching human milk.

During its 25 years of existence, CeSSIAM has been blessed with talented staff and students, dedicated collaborators and challenging research questions in the area of vitamins and minerals. Many opportunities remain to apply reliable scientific methods to new and important research questions in the future.

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