Center for Studies of Sensory Impairment, Aging, and Metabolism (CeSSIAM)

BULLETIN OF RESEARCH ABSTRACTS

Special issue on vitamin A

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CeSSIAM – Recent Publications (2012-2013)
Granada, tierra soñada por mí...

In his 1932 song, composer Augustín Lara portrays Granada as a land of dreams. Lara, of course, was not a Spaniard, but was born in Veracruz, Mexico. This immediately points out a fundamental characteristic of this city on a highland plateau in southern Spain; it has captivated an international imagination throughout the centuries. In unique and transcendental history, it was the epitome of a sense of “between nations”, as the capital of the Islamic Moorish culture, which reigned across the south of the Iberian Peninsula for 700 years from the early 8th century. Islamic domination and expansion continued until the 13th century when the Catholic elements from the north of Spain began a movement to take back the south of the peninsula.

Granada in international perspective

The year 1492 and the monarchs, Ferdinand of Aragon and Isabella of Castile, are pivotal for the history Granada and for all of what is now called Latin America. The armies coordinated by these Christian Monarchs defeated the Moors on January 2 of that year, completing the re-conquest (la Reconquista) of the Iberian Islamic domain of Al-Andalus (which then became Andalusia). Meanwhile, their investment in the exploration project of Christopher Columbus would pay its first dividend for Spain. On October 12 of that year, Columbus’ crew would sight the Caribbean island of Guanahani and herald the Iberian conquest (la Conquista) of the southern part of the Western Hemisphere. The Christian legacy of Spain provides the language and religion for most of Latin America. In Guatemala, we owe the tradition of the Holy Week processions to celebrations in the Canary Islands. The Moorish legacy is evident, however, every time we utter the expression “Ojala” (originating from the Arabic word, wa-šā’ Allāh = be it God’s will). The fiambre dish, consumed on the first of November in Guatemala, is said to originate from Moorish cuisine. The combination of cultures is memorialized in the Danza de los Moros y Cristianos (Dance of the Moors and Christians), with traditional masked dancers parading in the indigenous villages of the nation. It is a sight we have all seen in person, film or postcards.

For centuries Spain was host to Arabic culture and at the moment Granada keeps offering that Arabic feeling to its visitors. Living in Granada gives a sense of calm with really nice views like Sierra Nevada, Albaicín, the architecture of the Realejo buildings and the olive fields; but it also gives a sense of excitement. Granada has about 4 areas where people can go out until very late night, areas where you can see people of different ages or whole families enjoying a delicious meal and groups of friends having a “clara” (beer) or a glass of wine with a “tapa” or appetizer.

The sense of the city, even the decoration and organization of the city bars and restaurants changes with the weather, it can be really warm (44°C) in the summer, when restaurants and bars offer “Tinto de Verano” (a very refreshing drink prepared with wine and lime beverage). Weather can also be really cold (−5°C) in the winter, but the temperature is just perfect over the spring and autumn.

Granada is a city with large amounts of people coming from outside to study, due to its university history and good reputation it is known as a “students´ city”. Graná’inos, as people from Granada call themselves, are really friendly and helpful people, but do not expect to get their friendship and goodwill at the very first moment. Currently in Granada, the majority of the population is made up of young people, that gives the city an extra good vibe and nice feeling.

International Congress on Nutrition in Granada.

As important as the site of the XX International Congress of Nutrition in Granada, Spain, is the significance of international scientific meetings for the nutrition community and of the growing Ibero-American influence in contemporary times related to such meetings. Most of the CeSSIAM community, along with many from the Institute of Nutrition of Central America and Panama (INCAP) and the local universities, found their way to Havana, Cuba for the 16th Latin American Congress on Nutrition, the triennial gathering of the Latin American Nutrition Society (SLAN). This is deeply rooted in our region and language. What we learned was that, in a generic sense, the international meeting format is the true forum for networking and exchange that fosters the pollination and fecundity of a scientific discipline.

Prof. Angel Gil and the supporting Organizing and Scientific Committees of the XX ICN have elaborated an extraordinary Scientific Program for this coming September in Granada. Among its highlights are sessions on obesity throughout the life cycle and in relationship to food insecurity, prevention and management. Additional diseases and conditions in focus on the Program include HIV, cancer, inflammatory bowel diseases, food allergy and malaria. Food and cuisine traditions are projected in indigenous, Mediterranean and trans-European contexts. There
is strong focus on nutrition profiling and nutritional labeling by industry and evidence of its efficacy. This is extended with a symposium entitled: New Biomarkers for Health Claims Made on Food. There is exploration of micronutrient fortification, including biofortification and lipid-based nutrient supplements, and its monitoring and surveillance using new and innovative biomarkers for assessment. Lactation, maternal health and functional roles of human milk are covered in a series of programmed sessions. Advanced and innovative topics in nutritional science oriented around molecular genetics and genomics include: Dietary Reference Values for DNA Damage Prevention; Public Health Genomics in Individualized Nutrition; Personalized Nutrition in Medicine; and OMICS Technologies with Nutritional Perspectives.

The diverse thematic Task Forces of the IUNS system have their Symposia. Other entities with sponsorship of specific sessions include the World Health Organization (WHO), Food and Agricultural Organization (FAO), United Nations Children’s Fund (UNICEF) and the International Atomic Energy Agency from the United Nations System (IAEA), along with the National Institutes of Health (NIH) and the Center for Disease Prevention and Control (CDC) of the United States and the World Cancer Research Fund (WCRF), Helen Keller International (HKI) and Micronutrient Initiative. Additional offerings come from the COHORTS consortium, the HEALTHGRAIN project and the International Zinc Nutrition Consultative Group. Sessions focusing on specific regional and national needs are outlined for Europe, Asia, Latin America, the Middle East and North Africa as well as China and Vietnam. In addition, before and after the actual congress, Granada will be the site of Satellite Conferences and Symposia on topics ranging from bread and health and scaling-up nutrition to diet and the mental performance of children. This also includes International Food Data Conference and a satellite symposium on Sustainable Food and Diets.

**Ibero-American footprints moving forward**

The World Congresses Public Health Nutrition (WCPHN) have arisen from leadership of the Spanish Society of Community Nutrition, and have been held in one or another Iberian location in the years immediately following the last two ICNs: with the first edition in Barcelona, Spain in 2006, and second in Oporto, Portugal in 2010. The third WCPHN is planned for Las Palmas de Gran Canaria in the Canary Islands in 2014. In 2011, the European Nutrition Conference was held in Madrid, unfortunately for the two of us, fanatics of the FC Barcelona team, in the shadow of the Santiago Bernabéu Stadium.

However, when it came to the International Union of Nutritional Science, it has not been since the 8th ICN was held in Mexico City in 1972, that Spanish has been the language of the host nation. Forty-one years later, we shall gather in the shadow of the Alhambra and wander among the *Tapas* bars of Granada’s center. And four years from now, at the XXI ICN, we shall again be in the midst of a Spanish-speaking milieu among the *churrasquerías* and tango salons only blocks away from the River Plate, in Buenos Aires, Argentina.

**Concluding Remarks**

The clash of Christian and Islamic cultures, played out in *Al-Andalus* five centuries ago, is an unfortunate metaphor for contemporary world politics.

The theme of the ICN XX, “joining culture through nutrition,” is a refreshing antithesis – and potentially the antidote – to these poisonous currents. Science, with its basis in objectivity and rationality, and nutrition in particular, with its humanitarian purpose, represent the potential platforms to harmonize and integrate. With the winds from the SLAN congress at our backs, the CeSSIAM family looks forward to all of the information, networking and cultural harmonization that our hosts in Granada will afford in September.

María José Soto-Méndez & Noel W Solomons
Mounting and adaptation of a fluorescent rapid-assay device (iCHECK® FLUORO) for vitamin A in sugar and biological fluids
Zsofia Zambo, Jose David Sánchez-Mena, Mónica Orozco, Noel W Solomons

Guatemala was once a country with a high prevalence of low circulating retinol levels, that is <20 µg/dL. In the 1964-1965 Central American Survey of the International Committee for Nutrition in National Defense (ICNND), 26% of children under five years of age had retinol values below the cut-off criterion. This led to the mobilization of efforts at the Institute of Nutrition of Central America and Panama (INCAP) of Dr. Guillermo Arroyave for a method to add vitamin A to granulated table sugar in the national supply [1]. As part of the evaluation of the efficacy of fortified sugar, breast milk from lactating consumers was assayed for changes in vitamin A concentration [2]. Both of these studies in the 1970s depended on tedious and laborious extraction and colorimetric methods in the laboratory.

Analytical technology has advanced over the last three decades to allow for the emergence of rapid methods to assay food substances and biological fluids for their content of vitamin A. Prof. Florian Schweigert at the University of Potsdam in Germany conducted analytical chemistry, which led to a simplified system of solvents for extracting lipid-soluble compounds in a sealed vial. This has been manufactured and marketed by the BioAnalyt Company in Telbow, Germany as the iEX™ MILA vials. This extraction technology has been combined with a series of portable, compact battery operated devices for the analysis of nutrients in foods or biological fluids in a rapid manner and at the site of the collection, if warranted. One of these is the iCHECK FLUORO (BioAnalyt), which uses fluorescence as the analytic signal for quantifying retinol or retinyl esters. The developers in Potsdam collaborated with counterparts in Guatemala, to demonstrate the application of the iCHECK™ FLUORO system with the iEX™ MILA vials [3].

The present mission was to take the systems from a sophisticated university laboratory and adapt them to the a make-shift setting of a simulated field laboratory, while improvising the mixing and measuring equipment with items that can be purchased in pharmacies and supermarkets of low-income countries. In this exercise, we sought to prepare cow milk and heavy cream (as surrogates for human milk) and granulated sugar for rapid vitamin A analysis. A diverse array of plastic syringes was made for measurement instead of volumetric flasks or graduated cylinders. Clear plastic sandwich or storage bags were used for mixing, shaking and dilution. Spoons were substituted for spatulas to transfer sugar and medicine cups were tarred on the balance to weigh out sugar samples. The formal equipment, aside from the iCHECK™ device, were a digital balance (supplied by the manufacturer) and a Vortex mixer.

With respect to cow milk, 0.5 mL samples were delivered into iEX™ vials and, after agitation and re-sedimentation, the fluorescence measurements of the clear phase containing the extracted vitamin A were made in the well of the device. After 4 readings, a digital value in µg/L was given. Although the commercial milk’s label specified 900 µg/L, the average of 622 µg/L obtained by rapid assay was credible, given the notorious errors in dairy labeling of enriched nutrients. The coefficients of variation (CV) were 15% and 2.1%; inter-observer agreement of average measures was excellent. The concentration of vitamin A in heavy cream was so high, that the direct readings of 0.5 mL delivery exceeded the linear area of the device. When diluted to a suitable concentration, the average vitamin A concentration was 3536 µg/L.

For the preparation of solutions of table sugar, we used 20 mL plastic syringes, previously plugged with silicon to make them into a water-tight vessel. 4 g specimens of an unknown sugar sample were weighed to a 0.01 g precision on a digital balance (DigiWeigh, Chino, CA, USA). When filled to the syringe volume, a 20% (w/v) solution was obtained. For this dilution, a correction factor of 200 applied to the digital reading converts it into the concentration of vitamin A in the original sugar, as expressed mg/kg. Samples of different sugars were obtained from different brands, and were mixed in plastic bags to assure homogeneity. Nevertheless, CVs within sample for the observers ranged from 0.1-4.2%.

This exercise in adapting the iCHECK FLUORO system to a setting in a low-income country provided some lessons and conclusions. Inter-observer agreement for overall averages is high. The CVs within and between observers were high, especially for sugar. Assurance consistent values requires multiple replicate preparations, and this raises the costs, as the iEX™ MILA vials are a relatively expensive commodity.

References:

Zsofia Zambo is a masters degree student at the MacDonald College at McGill University in Quebec, Canada.

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Vitamin A content in sugar determined by a rapid assay device (iCHECK® FLUORO)
José David Sánchez-Mena, Zsofia Zambo, Mónica Orozco, Noel W Solomons

The public health success of the addition of vitamin A to granulated sugar was demonstrated during the first round of mandated fortification in the 1970s [1]. Given that Guatemala is a nation of stable fortification of table sugar, dating back to 1987, a system to monitor and confirm addition of vitamin A is required. A laboratory at the Institute of Nutrition of Central America and Panama (INCAP) has been using high precision liquid chromatography (HPLC) methods on sugar collected at sentinel sites to keep an ongoing control on the effectiveness of the fortification process. A rapid method to accomplish the same task – but with less complex and specialized techniques – would be welcome. The preceding abstract in this issue by Zambo et al. was the basis for this more concerted and systematic application of the iCHECK™ FLUORO system [2].

In this exercise, a total of 48 samples of sugar were collected from four, pre-established domain. This exercise reveals a number of lessons. It shows that the iCHECK™ system has strong promise to provide rapid and accurate information on the state of sugar fortification in Guatemala, Central America and any region that decides to protect its population with this measure.

After the two rounds of sugar sampling and rapid retinol analysis with iCHECK™ FLUORO documented in this issue of the Bulletin, it was pointed out that the degree of retinol dispersion within batches of fortified sugar in Guatemala raises issues about counting error that is consistent both with the inter-sample variation and the inter-observer agreement. The number of particles in 100 g of sugar is 60. Thus, there could be only 12 particles in the 4 g of sugar that we sampled, and the uneven distribution of just one retinol unit would change it by plus or minus 8%. By averaging multiple preparations of the same sample, one tends to get the correct median value, but counting-statistics would lead to any single value being too high or too low.

Despite this, among the 48 samples analyzed, 58.4% had an adequate vitamin A level, which is between 10–20 mg/kg of sugar. 29.2% were under-fortified, between 5–9.9 mg/kg. 8.3 had <5 mg/kg. Finally, 4.1% had an excess of vitamin A, that is >20 mg/kg.

Shown in the Table are the mean vitamin A values in the 48 specimens of sugar both by brand and origin and by geographic location of purchase. There were statistical difference in vitamin A content among the brands. The sugar bought in open sacks had, on average, half the vitamin A of the superior brand. On the other hand, there were no differences across the four region of origin.

One dilution regimen that could improve the counting statistics and improve the range of retinol concentration would be to weigh out 100 g of sugar, and dilute it to a one-liter volume. The down side of the trade-off, however, is the slower put through, and finding handy and disposable vessels that can be accurately filled to 1000 mL. Syringes of 100 mL volumes are the largest size commonly manufactured, generally used in veterinary medicine and cost up to 0.50 USD wholesale. This size was not located in Guatemala, but could be pre-ordered and imported. In this modification, we would weigh out 15 g of sugar, rather than 4 g, reducing the weighing error, but improving the counting error in replicate 15 g aliquots. Moreover, at a 15% (w/v) dilution, it would take a 20 ug/kg concentration in the sugar sample, which corresponds to upper limit of national mandate, and escape of a sample from the linear range of the instrument would be highly occasional.

However, even with the counting-statistics limitations of our present exercise, we can have confidence in the median values, since the error would be symmetrical. The iCHECK™ FLUORO system provided credible quantitative assessment of Guatemalan sugar, easily detected under-fortified and un-fortified samples and even allows differentiation among brands. It offers supreme promise for simplifying on-site monitoring of the sugar supply of Guatemala and other countries enrolled in the fortification movement.

References:

Table. Sugar vitamin A content by brand or origin and location of purchase

<table>
<thead>
<tr>
<th>Region of Purchase</th>
<th>Sugar vitamin A (mg/kg)</th>
<th>ANOVA by brand/origin</th>
<th>p = 0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metro Guatemala City</td>
<td>11.4±10.8ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greater Quetzaltenango</td>
<td>11.0±4.0ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigua Guatemala</td>
<td>11.8±4.7°ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Zone (Oriente)</td>
<td>8.4±5.6ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANOVA by region</td>
<td></td>
<td></td>
<td>p = 0.6003</td>
</tr>
</tbody>
</table>

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Noel W Solomons is scientific director of CeSSIAM
The assessment of vitamin A status is challenging and problematic. Vitamin A transport is homoeostatically regulated, making its measurement in biological fluids subject to distorted interpretation. This has implications for the diagnosis of hypovitaminosis A in the clinical setting, i.e. in a given patient, versus in the public health domain, i.e. in a given population. The only manner to determine how much vitamin A is in liver reserves of a specific individual would be using an expensive and cumbersome stable isotope test or performing a liver biopsy (either with a needle in a living patient or at autopsy in a deceased one). The measurements of circulating retinol levels, or retinol-binding protein levels, confront the problem that the release of retinol from its stores in the liver is based on the demand for the vitamin in the peripheral tissue. So, the blood levels do not go down until the liver is severely depleted. There is a general consensus that, at best, circulating vitamin A can be employed only in surveys to assess the status of a population, but not in a given individual [1]. Since breast milk can be obtained in a manner that is much less invasive and threatening than blood, it would be a preferred – if not ideal – biological fluid for population assessment, especially on the basis of the emerging rapid assessment technology illustrated in the literature [2] and in two previous abstracts in this issue.

Breast milk provides the vitamin A, both in the form of retinol and of provitamin A carotenoids, to supply the vitamin A needs of the nursing infant. The recommended “adequate intake” (AI) for infants in the United States and Canada is 400 μg/day during the first six postpartum months, and 500 μg/day during the second six months [3]. If we take the standard assumption of 780 mL for a full daily breast-milk volume for a healthy lactating mother, it would require an average concentration of 510 μg/L for the first trimester and 640 μg/L for the second trimester of life. International standards for adequate breast milk concentration, however, have been established at ≥300 μg/L [4]. The methodological brief by Schweigert et al [2] and their field experience with nomadic pastoralists in Chad in Africa [5], encouraged us to apply the technique of the new iCHECK FLUORO™ rapid assay among lactating rural women in Mam-speaking villages in the Western Highlands of Guatemala.

In the first phase of the study, we invited participants in the Mam-mamas study, who had given a sample of milk for the investigation when their infant had passed 5 mo of age to provide another specimen. A formal, standardized approach for “full breast extraction” was employed; this consisted of restrict nursing from a designated side, and having the infant nurse from the opposite side during 1.5 hours, with collection of all the milk in that “reserved” mammary gland with a bulb-operated breast pump. The milk was chilled on ice and later refrigerated. The concentration was measured on the same or next day in a single iEX™ vial extraction, with the measurement taken in triplicate. The average of three readings was assigned as the determined value. The coefficients of variation of replicate registration of vitamin A concentration was typically on the order of 2.01 – 2.52 %. In addition, the well-homogenated milk samples were centrifuged in capillary tubes in a hematocrit centrifuge to determine the volume of the cream fraction (Creamatocrit), expressed as a volume percent. Based on density relationships this could be transformed as well into an expression of percent milk fat in g/L. As retinol is a fat-soluble component, we expressed concentrations not only as the direct measurement, but also adjusted for creamatocrit and for milk fat concentration (see Tables). Finally, to better examine comparative issues of sensitivity and specificity, we created an additional category of milk retinol concentration, termed “marginal,” in the 300 to 399 μg/L range.

A total of 60 participants were enrolled from across seven Mam villages, with the number per village ranging from 3 to 17 lactating mothers. The individual concentrations ranged from a low value of 128 to a high of 1037 μg/L, with a mean of 508±202 μg/L and a median of 499 μg/L. A total of 15% of the sample had breast milk retinol values of <300 μg/L, whereas an additional 85% had values almost up to the maximum value of 1200 μg/L (based on European recommendations for infant formula, assuming 780 mL of daily consumption [5]), and no one had values above this superior limit. Excluding the smallest village, analysis of variance comparison of the mean milk retinols across the geographic sites found a significant inter-location difference (p=0.06).

In one of two studies, a within-subject differential sampling-technique exercise was conducted. In the routine practice of the study, a mixture of milk collection methods was applied to obtain samples, including manual expression from a partially-filled breast. Among 21 women, instructions were given to come to the formal extraction appointment with a manually-expressed sample from earlier in the same day (Table 1).
The mean retinol concentrations from the full-breast-collection series was 539±221 µg/L compared to that of casual, manual expression at 453±161 µg/L (p=0.154). Either method would give a comparable group estimate. Within-subject correspondence, as examined by the Spearman coefficient, however, was of low order, r = 0.053.

In the Schweigert et al [2] publication, the language of their comment “preliminary results suggest that this method works acceptably well in liquid and unfrozen samples of milk” raised our attention. We took this as an admonition not to rely on assays with the iCHECK™system if the sample has been frozen-stored and thawed prior to analysis. As this would severely compromise the field logistics in surveys, we sought to confirm or refute the notion that freezing of milk specimens distorts their measured retinol concentrations. In 21 full-breast samples, we made split-samples, analyzing one immediately on day of collection and freezing the second aliquot for from 21 to 26 days (Table 2). Upon comparison, the fresh sample had a retinol concentration was 539±221 µg/L compared to 528±205 µg/L after freezing (p=0.45). The Spearman correlation coefficient for the fresh versus the post-frozen assay was r = 0.96.

We can draw a number of conclusions from the findings in this study. The resources for an inter-method comparison with an HPLC standard were not available. Nevertheless iCHECK™ FLUORO and iEX™ system provides credible and reliable milk retinol values for human milk. A wide range of values are found, with 15% of samples having less than the international cut-off criterion of >300 µg on any given day. Since there is within-day variation in retinol concentration, this is unlikely to mean this rate would be seen consistently in a subset of women. Finally, at least short-term freezing for three weeks at -20°C, will not distort in any manner the individual or collect value for concentrations of retinol in stored samples.

Table 1. Mean vitamin A levels by method of milk collection in fresh milk: full breast vs. manual self-expression (n=21)

<table>
<thead>
<tr>
<th></th>
<th>Vitamin A normalized for creatancrit (µg/vol%)</th>
<th>Vitamin A normalized for milk fat (µg/g fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>539</td>
<td>453</td>
</tr>
<tr>
<td>SD</td>
<td>221</td>
<td>161</td>
</tr>
<tr>
<td>Median</td>
<td>499</td>
<td>464</td>
</tr>
<tr>
<td>Minimum</td>
<td>128</td>
<td>181</td>
</tr>
<tr>
<td>Maximum</td>
<td>1009</td>
<td>784</td>
</tr>
<tr>
<td>% low (&lt;300 µg/dl)</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>% marginal (300-399 µg/dl)</td>
<td>14</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2: Mean vitamin A levels for split-sample freezing experiment (n=21)

<table>
<thead>
<tr>
<th></th>
<th>Vitamin A normalized for creatancrit (µg/vol%)</th>
<th>Vitamin A normalized for milk fat (µg/g fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full breast - fresh</td>
<td>Full breast - frozen</td>
</tr>
<tr>
<td>Mean</td>
<td>539</td>
<td>528</td>
</tr>
<tr>
<td>SD</td>
<td>221</td>
<td>205</td>
</tr>
<tr>
<td>Median</td>
<td>499</td>
<td>509</td>
</tr>
<tr>
<td>Minimum</td>
<td>128</td>
<td>152</td>
</tr>
<tr>
<td>Maximum</td>
<td>1009</td>
<td>948</td>
</tr>
<tr>
<td>% low (&lt;300 µg/dl)</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>% marginal (300-399 µg/dl)</td>
<td>14</td>
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</table>

References

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Guatemala is the country in which the technique for fortification of granulated table sugar was developed and in which population-wide vitamin A fortification was first implemented (1). The vitamin A requirement for women in the non-reproductive state is 500 retinol activity equivalents (RAE) and increases considerably to 800 RAE (a 60% increase) during pregnancy, and to 850 RAE (a 70% increase) during lactation (2).

On the one hand, this represents a major challenge for dietary behavior, unless overly abundant vitamin A is being consumed from the customary diet. On the other hand, the period immediately following conception is the period of embryonic vulnerability to excess exposure to retinoids. An upper tolerable intake level (UL) of 3000 µg of preformed vitamin A was established, based on extrapolation from the study by Rothman et al (2). However, still controversial literature, suggests that chronic consumption of preformed vitamin A at levels beginning as low as 1500 µg/d, may produce demineralization of the skeleton and increase susceptibility of osteoporotic fracture (3).

With concerns that vitamin A intakes by reproductive-stage mothers exposed to the Guatemalan sugar-fortification policy may have consequences from persistent underconsumption of the vitamin to intakes on the excessive side of the normative distribution, we focused our analysis on specific sources and total intake of vitamin A. We present here the results of our analysis examining eating behavior in a small sample of pregnant and lactating women in the interior of Guatemala.

Convenience samples of 181 women, both pregnant and lactating, were recruited in three settings – urban, semi-urban and rural – in the Province of Quetzaltenango in the Guatemalan Highlands. A single, previous-day dietary recall for each participant was collected. An adjusted USDA food composition table was used to calculate total vitamin A, pro-vitamin A and preformed vitamin A intakes. A standard fortification level of 10 µg/g was assumed for sugar fortification. The contribution from fortified granulated sugar, other fortified foods such as Incaparina®, natural preformed sources and provitamin A carotenoids were calculated. The proportion of women with estimated intakes below the recommended nutrient intakes (RNI) and above the upper tolerable level were evaluated.

Total 1-day vitamin A intake estimates were between 1021 and 1318 RAE in the urban and semi-urban areas, and much lower at just under 600 RAE in the rural area for both pregnant and lactating women (table 1). In the metropolitan area, preformed vitamin A (plant and mixed sources) intake was higher than preformed vitamin A (sugar and non-sugar derived) intake, whereas in the rural area the opposite was true. Median sugar intake was 51 g delivering 512 RAE units in the metropolitan area, and 25 g delivering 249 RAE units in the rural site (table 2). The highest sugar intake was 207 g, delivering 2073 RAE units. The importance of fortified food sources for total vitamin A is illustrated in table 3, in which cumulative contributions of dietary vitamin A from sugar fortification, other fortified food products, natural sources of preformed vitamin A and provitamin A carotenoids are illustrated.

The proportion of women with intakes below the RNI was 21%, 31%, and 53% for pregnant women from the urban, semi-urban and rural sites, respectively, and 22%, 26%, and 87% for lactating women from the urban, semi-urban and rural sites, respectively (figure 1). Four pregnant women from the semi-urban area, two lactating from the urban and two lactating from the semi-urban area had estimated preformed vitamin A intakes above 1500 µg and a single lactating women from the urban area had intakes above 3000 µg.

We conclude that fortified granulated table sugar makes an important contribution to vitamin A intake amongst all women in this sample. Without the contribution of this source only 25% of pregnant and lactating women would have met their requirement. In addition other sources of preformed vitamin A such as Incaparina® and corn flakes and a natural source in eggs represent the next most important contribution to total vitamin A intake in women living in the urban and semi-urban areas. Provitamin A sources constitute less than 20% in contrast to the rural women where plant sources contribute over 40% of vitamin A intake. As such, neither the metropolitan nor the rural women have desirable balance of vitamin A sources in the diet.

On the one side, a still big group of women, especially those living in the rural areas, do not meet their status specific RNI when in the reproductive phase of their lives. On the other side, a total of 10 women had preformed vitamin A intake in women living in the urban and semi-urban areas. Provitamin A sources constitute less than 20% in contrast to the rural women where plant sources contribute over 40% of vitamin A intake. As such, neither the metropolitan nor the rural women have desirable balance of vitamin A sources in the diet.

We conclude that fortified granulated table sugar makes an important contribution to vitamin A intake amongst all women in this sample. Without the contribution of this source only 25% of pregnant and lactating women would have met their requirement. In addition other sources of preformed vitamin A such as Incaparina® and corn flakes and a natural source in eggs represent the next most important contribution to total vitamin A intake in women living in the urban and semi-urban areas. Provitamin A sources constitute less than 20% in contrast to the rural women where plant sources contribute over 40% of vitamin A intake. As such, neither the metropolitan nor the rural women have desirable balance of vitamin A sources in the diet.

References
Table 1. Estimated median 1-day total, pro- and preformed vitamin A intakes in pregnant and lactating women by study setting

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women</th>
<th>Lactating women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban (n=29)</td>
<td>Rural (n=30)</td>
</tr>
<tr>
<td>Total vitamin A (RAE)</td>
<td>1021</td>
<td>591</td>
</tr>
<tr>
<td>Total provitamin A (plant and mixed sources) (RAE)</td>
<td>127</td>
<td>236</td>
</tr>
<tr>
<td>Total preformed vitamin A (sugar and non-sugar derived) (µg)</td>
<td>912</td>
<td>277</td>
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Table 2. Cumulative contributions of dietary vitamin A in RAE units from diverse dietary sources

<table>
<thead>
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<tr>
<td></td>
<td>Urban area (n=29)</td>
<td>Semi-urban area (n=29)</td>
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<tr>
<td>Vitamin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar fortification</td>
<td>533</td>
<td>46</td>
</tr>
<tr>
<td>Other fortification</td>
<td>348</td>
<td>50</td>
</tr>
<tr>
<td>Natural preformed</td>
<td>54</td>
<td>5</td>
</tr>
<tr>
<td>Provitamin A carotenoids</td>
<td>224</td>
<td>19</td>
</tr>
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</table>

Table 3. Estimate 1-day intake of table sugar and vitamin A contribution from this sugar

<table>
<thead>
<tr>
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<th>Lactating women</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Urban (n=29)</td>
<td>Rural (n=30)</td>
</tr>
<tr>
<td>Granulate table sugar fortified with vitamin A</td>
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<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>12</td>
<td>75</td>
</tr>
<tr>
<td>25th percentile</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>Median</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td>75th percentile</td>
<td>69</td>
<td>68</td>
</tr>
<tr>
<td>Maximum</td>
<td>207</td>
<td>147</td>
</tr>
<tr>
<td>Vitamin A contribution from granulated table sugar (RAE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>124</td>
<td>75</td>
</tr>
<tr>
<td>25th percentile</td>
<td>241</td>
<td>262</td>
</tr>
<tr>
<td>Median</td>
<td>470</td>
<td>477</td>
</tr>
<tr>
<td>75th percentile</td>
<td>689</td>
<td>677</td>
</tr>
<tr>
<td>Maximum</td>
<td>2073</td>
<td>1469</td>
</tr>
</tbody>
</table>

Figure 1. Proportion of women with estimated intakes below the RNI or above the upper tolerance level

Ingrid Bielderman is an exchange student at CeSSIAM during her MSc degree at Wageningen University.
Deborah Fuentes is a research nutritionist at CeSSIAM-Quetzaltenango.
Elena Maria Díaz Ruiz is a research nutritionist at CeSSIAM-Quetzaltenango.
Marieke Vossenaar is a research nutritionist at CeSSIAM.
Noel W Solomons is the scientific director at CeSSIAM.
As discussed, Guatemala has been in the forefront of the permanent control and prevention of vitamin A deficiency by way of a sugar fortification. As of the most recent national survey of retinol levels in under-five children, only 3% had a concentration of <20 µg/dL [1]. This is indicative of the total absence of a hypovitaminosis A as a public health problem in Guatemala. The only remaining question would be whether or not some individuals have excessive exposure to preformed dietary vitamin A due to abundant consumption of sources, including fortified sugar.

The study was conducted as part of a larger research project, undertaken in a total of 12 small communities in rural areas on the southern coast of Guatemala. It involved families who were engaged during part of the year in the agro-industry of sugar-cane working with the harvesting of the sugar-cane crop or the milling and refining of the material into the finished, refined granulated sugar. They were beneficiaries of the social programs of the charity of the sugar industry, FUNDAZUCAR. Blood samples were drawn in the month of March, 2011. The sub-groups were constituted of apparently healthy and non-diabetic adult women in the fertile age (18 – 48 y, n=157) and comparable school children (aged 6-11 y, n=60 boys and n=74 girls). Plasma samples were collected, frozen and delivered to the laboratories of the Institute of Nutrition of Central America and Panama (INCAP). Total circulating retinol was measured with high precision liquid chromatography. In addition, since circulating vitamin A levels are susceptible to distortion by active inflammation, biomarker of inflammation – C-reactive protein (CRP) and alpha-1 globulin (AGP) – were measured in the same samples in the same laboratory. We used cut-off values to classify the adequacy of intake based on classification criterion that technically refer to surveys of children aged 6 to 59 mo, these were: <10 µg/dL deficient, 10-19.9 and 10 µg/dL low, 20-29.9 µg/dL marginal, and ≥30 µg/dL adequate.

The descriptive statistics are shown in Table 1. We measured levels of inflammatory biomarkers, CRP and AGP, and applied a correction procedure for depression of circulating retinol concentration suggested by David Thurnham and colleagues [2]. To be clear, the mathematical procedure was devised to examine the lower tail of a retinol concentration distribution and to see if any over-diagnosis of low levels is being made. When the correction factor was applied to the retinol values of the 58 samples indicating inflammation, the average for women’s retinol was increased by 4% and that for school-children was increased by 2%.

The distribution of retinol for groups other than young children cannot be used to assess the public health status of a population in any official way. However, as a convenience sample of two age groups, our results confirm the general absence of low levels. Compared with circulating vitamin A values from adult women and schoolchildren of different ethnic groups from the U.S. National Health and Nutrition Examination Survey (NHANES) [3,4], the values on the southern coast were up to 50% higher than the North American reference levels.

References:
4. Looker AC, Johnson CL, Woteki CE, Yetley EA, Underwood BA. Ethnic and racial differences in serum vitamin A levels of different ethnic groups from the U.S. National Health and Nutrition Examination Survey (NHANES) [3,4], the values on the southern coast were up to 50% higher than the North American reference levels.

Table 1. Descriptive statistics of plasmatic retinol values

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schoolchildren (n=134)</td>
<td>45.9±14.1</td>
<td>42.5</td>
<td>27.1-125.8</td>
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<tr>
<td>Women (n=157)</td>
<td>61.8±17.7</td>
<td>57.4</td>
<td>27.8-128.2</td>
</tr>
<tr>
<td>Total (n=291)</td>
<td>54.5±17.5</td>
<td>51.0</td>
<td>27.1-128.2</td>
</tr>
</tbody>
</table>

Noel W Solomons is the scientific director of CeSSIAM
Raquel Campos is a research nutritionist at CeSSIAM
María-José Soto-Méndez is a research nutritionist at CeSSIAM and a doctoral student at the Universidad de Granada
Carolina Martínez is a research scientist at the Institute of Nutrition of Central America and Panama (INCAP)
In 2012, the Hildegard Grunow Foundation reached five years of age. It was founded to honor a wonderful mother, mother-in-law, grandmother and community citizen. She was born in 1923 in Dortmund, as one of 3 children of the geologist and mathematician Dr. Alfred Brune, who worked for coal-mining company in the highly industrialized Ruhr area. Leaving school during the war she was trained as a medical technician and worked in physiological research at a Kaiser Wilhelm Institute in 1944/45. In the baby-boomer years after the war she married a merchant in Hamburg with whom she had two children. After her divorce in the late 1950’s, she married a lawyer in Hamburg who died 8 years later. The two marriages had left her with ample financial means of which she used up very little due to her modest life style. Her heritage formed the fundament of the Hildegard Grunow Foundation which was founded in August 2007. Sparked by her own early work in physiology, by the experience of hunger and hardship during the war, as well as by the death of her second husband on the sequels of “metabolic syndrome” and “stress-disease” she agreed with her son to make work on the underlying causes of nutrition-related pathologies the main focus of Hildegard-Grunow-Foundation. Today, in a small office at 27 Clemensstrasse in the Schwabing neighborhood of Munich, the various tasks of a charitable foundations are carried out by a single, part-time staff coordinator, Ms Gabi Boerries.

The origins of the HGF come out of a matter of serendipity. Noel Solomons a physician intestinal physiologist and Klaus Schümann a physician toxicologist, both identified with the micronutrient nutrition community, began a collaboration after meeting at a scientific meeting on nutrient biological availability in Ede, the Netherlands in 2000, riding on a train from Cologne to Mannheim in Germany. The idea was to test the ability of heme iron to serve as a fortificant to improve iron status as a potentially more biologically available and less oxidizing form of iron. Over the next two years, discussions led to a multi-institutional collaboration among Pennsylvania, Innsbruck, Freising, Munich and Guatemala and a field trial in anemic children on the outskirts of Guatemala City. The heme iron was added to a Guatemalan staple food, mashed black beans.

During this same period, Solomons and Schümann found a kindred spirit in a concern for a safer approach to the use of oral iron in Dr. Rainer Gross. Gross’ approach was to give iron only once or twice weekly, and we participated in his consultation meetings in Brazil and Peru, which guided his landmark International Research on Infant Supplementation (IRIS) trials, which tested the approach. Clearly, momentum was building for safer oral iron administration. The findings of the heme-iron study were published in 2005 in the journal, Public Health Nutrition. In the same year in Toxicology, a paper would appear on daily oral iron supplementation on biomarkers of oxidation in healthy, young volunteers, product of a German graduate student exchange, in collaboration with the CeSSIAM staff and international collaborators. Simultaneously, a third in the publication, Journal of Pediatric Gastroenterology and Nutrition, on iron, copper and zinc in human milk from Guatemalan mothers, by two masters students from Wageningen University.

So, with these antecedent activities and with some of the inheritance from the late Mrs. Grunow, in August 2007 in Munich, Germany, a non-profit foundation was founded in her name. The mission statement states: “Its purpose is to promote and sponsor research in the fields of nutrition and food science, as applied to public health and medicine, including molecular, toxicological and physiological aspects, as well as ecology and conservation of natural resources. One focus of the Foundation is on the interaction between nutrition, health and ecology, whereas another is on training of young scientists and health professionals and local capacity building of emerging institutions, both focused preferentially in the developing world.”

In terms of specific research investment, areas of iron safety have been prioritized. This has covered mounting and applying assays, such as that for the non-transferrin-bound iron (NTBI) and for anti-oxidant quenching of fecal free radicals, which can address the hazards of oral administration of iron. HGF has invested time and funding into solving the dilemma of oral iron administration in areas with a high transmission of malaria. This includes non-invasive screening to detect anemia, and modifying the chemical nature and timing of iron supplementation to reduce its accumulation in the circulation. On the capacity-building front, the HGF has provided fellowship support to two international masters students from the Technische Universitaet Muenchen, Juliana Casimiro de Almeida of Brazil and Carolina Luzon of Peru for their thesis research exchange to Guatemala. At the level of doctoral studies, HGF is supporting Maria Jose Soto-Mendez of the Guatemala staff.

Another initiative of the HGF has been to commemorate the life and legacy of our departed friend, Rainer Gross, the Rainer Gross Prize: Recent Issues in Nutrition and Health. Every two years, beginning in Oporto, Portugal in 2010 and in 2012 in Havana, Cuba, this prize has been awarded, in the first opportunity to Aaron Lechtig of Peru and Angela Cespedes of Bolivia, and on the second, to Manuel Ruz of Chile and Kenneth Brown of the USA. The awards lectures are published in the Food and Nutrition Bulletin.

CeSSIAM and the HGF are examples of parties in a symbiotic relationship in the most profound connotation of the term. If it were not for the personnel and research mission of the Center in Guatemala, the Foundation is unlikely to have existed. Were it not for the resources and support provided to CeSSIAM by the HGF, its website would not be posting the growing list of publications. We look forward to seeing how much of the mission will have been completed when we come to our 10th anniversary.

Klaus Schümann, Gabi Boerries, Noel W Solomons & Klaus Naeve
2012


2013


Oyesiku L, Doak CM, Solomons NW, & Vossenaar M. Highland Guatemalan women are extremely short of stature and no lactation-duration effects on body composition are observed in a cross-sectional survey. *Nutrition Reviews* 2013;71(2):87-94.


