
Mass Spectrometry 2017: Natural abundance of ^{13}C in serum retinol differentiates between dietary intakes of C3 versus C4 plants - Sherry A Tanumihardjo - Pennsylvania State University**Abstract**

Vitamin A is a micronutrient essential in vision, reproduction, immune function, and cellular differentiation. Provitamin A carotenoids are plant sources of vitamin A. The isotopic distribution of ^{13}C and ^{12}C in humans is determined by what foods are consumed. C3 plants, i.e., green vegetables, carrots, and pumpkins, have lower $^{13}\text{C}:^{12}\text{C}$ than C4 staple crops, i.e., maize, sorghum, and millet. Vitamin A foods from corn-fed animals will reflect the $^{13}\text{C}:^{12}\text{C}$ feed that the animals eat. The serum retinol $^{13}\text{C}:^{12}\text{C}$ was previously evaluated as a biomarker for vegetable intake. The retinol $^{13}\text{C}:^{12}\text{C}$ decreased in humans who increased their vegetable intake (range -26.21 to -31.57% , $P = 0.050$) and correlated with provitamin A carotenoid intake ($P = 0.079$). The average δ difference was -0.526 with increased vegetable intake, while control increased by $+0.370$. A $2 \times 2 \times 2$ study in Mongolian gerbils fed white and orange maize or carrots for an extended period of time. Serum retinol $\delta^{13}\text{C}\%$ differentiated between those consuming white maize and white carrots ($-27.1 \pm 1.2 \delta^{13}\text{C}\%$) from those consuming orange maize and white carrots ($-21.6 \pm 1.4 \delta^{13}\text{C}\%$, $P < 0.0001$) and white maize and orange carrots ($-30.6 \pm 0.7 \delta^{13}\text{C}\%$, $P < 0.0001$). This method was applied to Zambian children who had been fed either orange or white maize for two months. Those children who consumed orange maize had a lower $\delta^{13}\text{C}\%$ (-26.64 ± 1.98) than their

white maize-consuming counterparts (-27.39 ± 1.94) ($P = 0.049$). In the application of this methodology to efficacy or effectiveness trials, it will be important to choose the appropriate control group and number of subjects for comparison analyses.

Objectives: We investigated changes in serum retinol relative differences of isotope amount ratios of $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$) caused by natural ^{13}C fractionation in C3 compared with C4 plants as a biomarker to detect provitamin A efficacy from biofortified (orange) maize and high-carotene carrots.

Keywords: biofortification, stable carbon isotope, vitamin A+, carrot, GCCIRMS, vitamin A effectiveness, vitamin A efficacy.

Introduction

Biofortifying staple and horticultural foods with provitamin A carotenoids can sustainably ensure adequate vitamin A (VA) intakes and mitigate potential hypervitaminosis A risks caused by preformed VA in high-dose supplements and fortified foods. The bioefficacy of high provitamin A (orange) maize, defined as the production of retinol from consumed provitamin A carotenoids, has been demonstrated in gerbil studies and single-meal feeding studies in humans. To evaluate health-promoting interventions in humans, efficacy and effectiveness trials are

conducted. Efficacy trials are characterized by ideal circumstances that maximize the likelihood of observing a treatment effect: a selected homogeneous population, standardized intervention, and experienced providers or study facilitators. Effectiveness trials are characterized by real-world circumstances designed to determine whether the intervention works as actually used or adopted: a broad heterogeneous population, less standardized treatment protocols, and usual providers. A human bioefficacy study determined that orange maize (OM) is an efficacious VA source in children, but to our knowledge effectiveness trials have not yet been carried out.

Liver VA concentration is the gold standard for evaluating VA status; however, this is only feasible in animal studies or in special cases in humans. Serum retinol concentrations are homeostatically controlled over a wide range of liver reserves (12214), are affected by infection and inflammation, and are nonsensitive indicators of changes in VA status. Furthermore, several indicators used for VA assessment, such as serum retinol and dose-response tests, are qualitative because they only distinguish deficiency from adequacy. Studies performed for provitamin A-biofortified maize in humans have used multiple blood draws for postprandial response or stable isotope methods with intrinsically labeled maize and tracer VA doses for isotope dilution. These techniques require dosing and multiple blood samples per subject, which may not be practical in large-scale effectiveness studies, particularly in children.

Most plants used for food, including staples such as wheat and rice, are C3 plants; however, there are a few notable crops used for human consumption that are C4 plants (e.g., maize, millet, sorghum, sugar cane).

C3 plants discriminate more against ^{13}C during photosynthesis and therefore have lower ^{13}C enrichment than C4 plants (18). ^{13}C content at natural abundance concentrations is often expressed using the δ notation, which refers to Vienna Pee Dee Belemnite (VPDB) and is expressed as $\delta^{13}\text{C} = \left[\frac{R_{\text{sample}}}{R_{\text{VPDB}}} - 1 \right] \times 1000$; $R = ^{13}\text{C}/^{12}\text{C}$ (19). This value is then typically expressed per mil (‰) by multiplying by 1000. VPDB is relatively enriched compared to most natural materials and has been assigned a $\delta^{13}\text{C}$ value of 0; therefore, most other natural materials have negative $\delta^{13}\text{C}$ values. Atmospheric CO_2 is relatively stable geographically and topographically and has reported $\delta^{13}\text{C}$ values ranging from 27.4‰ to 26.7‰ (20). C4 plants typically have $\delta^{13}\text{C}$ values closer to atmospheric CO_2 [e.g., maize (213.6‰ and 214.0‰) (18), sorghum (213.8‰ and 214.4‰) (18), and millet (210.7‰ and 212.0‰) (21)]. C3 plants have lower $\delta^{13}\text{C}$ [e.g., carrots (29.5‰ and 60.2‰), bananas (226.6‰ and 60.1‰), and mangos (225.4‰ and 60.1‰) (17)]. Lipids and other secondary metabolites (including carotenoids) are further reduced in $\delta^{13}\text{C}$ by 5210‰ (22, 23); however, the difference between C3 and C4 plants is maintained, as noted with lutein obtained from marigold compared with maize (229.9‰ and 60.2‰ and 219.8‰ and 60.3‰, respectively) (23). This difference gives the potential to in vivo metabolites in determining dietary plant origins based on ^{13}C composition. Because no carbon is gained or lost during the cleavage of b-carotene or other provitamin A carotenoids to VA, the $\delta^{13}\text{C}$ of serum retinol can reflect the dietary sources, including preformed and provitamin A. The principle of isotope mass balance states that the amount of heavy isotope in a system is a linear combination of its components (19), which could be used to quantitatively estimate the relative contributions of dietary vitamin A sources.

Several C4 crops are being biofortified with provitamin A carotenoids; in addition to maize, sorghum and millet are also targets. C4 plants often have advantages over C3 plants under conditions of drought, heat, and CO₂ or nitrogen limitations, and for this reason they are major crops in tropical and subtropical regions. Furthermore, they may play a vital role in food and nutrition security under changing climates. These biofortified varieties should be confirmed for VA bioefficacy and effectiveness at the population level.

This controlled study was undertaken to determine whether b-carotene efficacy from

OM could be demonstrated with the use of shifts in the $\delta^{13}\text{C}$ of serum retinol from the natural enrichment of maize feeding and comparing these values to the $\delta^{13}\text{C}$ of liver VA, liver VA concentrations, and serum retinol concentrations. The $\delta^{13}\text{C}$ was determined with GC combustion isotope ratio MS, which is known for its high degree of precision at natural abundance concentrations (30). Mongolian gerbils are a useful model for human absorption and metabolism of provitamin A carotenoids (31233). Findings in maize could also extend to millet and sorghum because of their similar ^{13}C enrichment.

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