Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: erythrocyte incorporation of iron and apparent absorption of zinc, copper, calcium, and magnesium from a complementary food based on wheat and soy in healthy infants\textsuperscript{1–3}

Lena Davidsson, Ekhard Ziegler, Christophe Zeder, Thomas Walczyk, and Richard Hurrell

ABSTRACT

Background: Phytic acid is a strong inhibitor of iron absorption from fortified foods. In adults, this inhibitory effect can be overcome by adding ascorbic acid with the iron fortificant or by using a “protected” iron compound such as NaFeEDTA. In addition, the use of NaFeEDTA as an iron fortificant has been reported to increase zinc absorption in adult women. No information is available on iron bioavailability from NaFeEDTA or the influence of NaFeEDTA on minerals and trace elements in infants.

Objective: We aimed to compare iron bioavailability from a complementary food based on wheat and soy fortified with either NaFeEDTA or ferrous sulfate plus ascorbic acid. The apparent absorption of zinc, copper, calcium, and magnesium was evaluated in parallel.

Design: Stable-isotope techniques were used in a crossover design to evaluate erythrocyte incorporation of iron 14 d after administration of labeled test meals and the apparent absorption of zinc, copper, calcium, and magnesium on the basis of fecal monitoring in 11 infants.

Results: Geometric mean erythrocyte incorporation of iron was 3.7% (NaFeEDTA) and 4.9% (ferrous sulfate plus ascorbic acid) ($P = 0.08$). No significant differences in the apparent absorption of zinc, copper, calcium, or magnesium were observed between test meals ($n = 10$).

Conclusions: Iron bioavailability from a high-phytate, cereal-based complementary food fortified with either NaFeEDTA or ferrous sulfate plus ascorbic acid was not significantly different. NaFeEDTA did not influence the apparent absorption of zinc, copper, calcium, or magnesium. NaFeEDTA does not provide any nutritional benefit compared with the combination of a highly bioavailable iron compound and ascorbic acid. *Am J Clin Nutr* 2005;81:104–9.

KEY WORDS Iron compound, food fortification, stable isotopes, infant cereal

INTRODUCTION

Iron fortification of complementary foods has long been implemented as a public health strategy in industrialized countries to combat iron deficiency during early life. However, some concern exists about the efficacy and effectiveness of iron-fortified complementary foods for preventing iron deficiency in infants and young children. A major problem related to the potential effect of iron-fortified complementary foods such as infant cereals is that unacceptable organoleptic changes may occur during storage or during food preparation of fortified products containing water-soluble iron compounds with high relative bioavailability. Consequently, non-water-soluble iron compounds are often used to fortify infant cereal products, although some of the most commonly used iron compounds have been shown to have low relative bioavailability and can therefore be expected to have only a limited effect on the iron status of the consumers (1, 2). Furthermore, it is important to note that the bioavailability of iron compounds used in food fortification programs is dependent on the presence of enhancers and inhibitors in the diet. Thus, iron absorption from highly bioavailable iron compounds can be low from products based on cereals and soy because of the presence of phytic acid (3, 4).

Ascorbic acid has been shown to be a potent enhancer of iron absorption in both adults and children (4–10), and this vitamin is therefore often added during the manufacture of industrially produced complementary foods to counteract the inhibitory effect of phytic acid. However, losses of ascorbic acid during processing, storage, and food preparation might limit the usefulness of this approach in some settings. As an alternative to ascorbic acid, Na$_2$EDTA has been evaluated as an enhancer of iron bioavailability in adults and schoolchildren. Although this strategy was shown to be useful to increase iron absorption from ferrous sulfate added to low-bioavailability meals (11–14), no enhancing effect was found when Na$_2$EDTA was added to meals fortified with ferrous fumarate (15, 16).

Clearly, the use of an iron compound with high relative bioavailability whose absorption is not susceptible to the negative effects of inhibitory ligands would be a useful way of providing...
iron via fortified foods. NaFeEDTA is a water-soluble iron compound that is less influenced by the presence of phytic acid (13, 15, and reviewed by the International Nutritional Anemia Consultative Group in reference 17). In addition, we reported significantly higher apparent zinc absorption in women, but no effect on calcium absorption, from bread fortified with NaFeEDTA than when ferrous sulfate was used as a food fortificant (18). However, no information is available on iron bioavailability from NaFeEDTA or the influence of NaFeEDTA on minerals and trace elements in infants.

The aim of the present study was to compare iron bioavailability in healthy infants from a complementary food based on wheat and soy and fortified with either NaFeEDTA or an iron compound with high relative bioavailability (ferrous sulfate) under optimal conditions (ie, in the presence of ascorbic acid). Erythrocyte incorporation of iron stable isotopes 14 d after administration was used as a proxy for iron absorption. In parallel, the apparent absorption of zinc, copper, calcium, and magnesium was evaluated by use of a stable-isotope technique based on fecal monitoring.

SUBJECTS AND METHODS

Eleven healthy infants (5 boys and 6 girls, 18–27 wk old) were recruited to allow for one dropout. All eleven infants were fully informed about the aims and procedures of the study, and written consent was obtained from at least one parent of each infant. The study protocol was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects.

The sample size was based on previous data on erythrocyte incorporation of iron stable isotopes in infants (4). It was estimated that 10 infants would be a sufficient sample size to detect a nutritionally significant difference in erythrocyte incorporation of 50% with 90% power and a type I error rate of 5%. Eleven infants were recruited to allow for one dropout. All eleven infants completed the iron absorption study. Ten infants completed the metabolic balance study.

Study design

The study used a balanced, crossover design to evaluate erythrocyte incorporation of iron and the apparent absorption of zinc, copper, calcium, and magnesium from test meals fortified with either NaFeEDTA or ferrous sulfate (FeSO₄). Each study consisted of the ingestion of 2 isotopically labeled test meals followed by the collection of fecal material for 72 h. Capillary blood samples were drawn before and 2 wk after ingestion of the test meals. The second blood sample of the first study was used as the baseline sample for the second study. Test meals were served in a predetermined and random order (NaFeEDTA-FeSO₄ or NaFeEDTA). Infants were fed 1–2 servings/d of a commercial iron-fortified infant cereal (Ceresoy; Nestlé, Vevey, Switzerland) that was similar to the test meal for 2–3 wk before the start of the study. The prefeeding period was included in the protocol to ensure acceptance of the cereal product by the study infants.

Test meals

An infant cereal based on wheat flour and soy flour was produced especially for this study without any added minerals or vitamins at a Nestlé Product Development Center (Linor, Orbe, Switzerland). Each test meal consisted of 20 g cereal reconstituted with 60 g hot ultrapure water. Labeled test meals were fed after the infants had fasted overnight, or ≥3 h after intake of infant formula, under standardized conditions on day 1 of each study. Stable-isotope labels of iron, zinc, and calcium were added to the first test meal. The second test meal was labeled with stable isotopes of copper and magnesium. The total content of added iron, zinc, and calcium was equilibrated in the second test meal by the addition of minerals with normal isotopic composition. Doses of stable-isotope labels were 888 μg 70Zn (ZnCl₂), 1.0 mg 65Cu (CuCl₂), 5 mg 25Mg (MgCl₂), and 4 mg 44Ca (CaCl₂). Iron was added as 2.0 mg 58Fe (FeSO₄) or 2.0 mg 58Fe (FeCl₃) mixed with Na₂EDTA as an aqueous solution in a 1:1 molar ratio (Fe:EDTA). Fe:EDTA solutions were prepared immediately before administration was used as a proxy for iron absorption. In parallel, the apparent absorption of zinc, copper, calcium, and magnesium was evaluated by use of a stable-isotope technique based on fecal monitoring.

Stable-isotope labels

Highly enriched stable isotopes of 70Zn, 65Cu, 25Mg, 44Ca, and 58Fe were purchased from a commercial supplier (Isotec, St-Quentin, France) as metals and were converted into 70ZnCl₂, 65CuCl₂, 25MgCl₂, 44CaCl₂, 58FeSO₄, and 58FeCl₃, respectively.
All solutions were diluted with ultrapure water (18 MΩ; Millipore Super Q, Bedford, MA), and individual doses were filled into acid-washed polytetrafluoroethylene vials, flushed with argon, and kept refrigerated until used. Isotopic composition was determined by thermal ionization mass spectrometry [(TIMS) MAT 262; Finnigan MAT, Bremen, Germany].

Sample preparation and analysis

Because of the high risk of contamination during mineral and trace element analysis, special care was taken during sample handling, preparation, and analysis. Only acids purified by sub-boiling distillation and ultrapure water (18 MΩ, Millipore Super Q) were used for the preparation of stable-isotope solutions and for all analytic work. Other chemicals were of analytic grade purity. To minimize contamination through vessel materials, only acid-washed quartz, polytetrafluoroethylene, and polyethylene containers were used. Powder-free gloves were used during all sample handling and analysis.

Fecal material and blood samples were shipped on dry ice to Rüschlikon, Switzerland, for analysis. Fecal material was freeze-dried, ground to a fine powder in acid-washed mortars, and pooled into 72-h pools before further analysis. Fecal pools included the first fecal sample dyed by carmine red and all consecutive stools up until, but not including, fecal material dyed by the second dose of carmine red. Results from a previous study (20) confirmed that 72 h is a sufficient time period for complete collections of unabsorbed stable isotopes in fecal material in infants consuming infant cereal.

Blood samples

Blood samples were analyzed in duplicate under chemical blank monitoring. Samples of whole blood (0.5 mL) were mineralized in a mixture of 5 mL concentrated HNO₃ and 2 mL 30% H₂O₂ in tetrafluoroethylene-perfluoro(alkoxy vinyl ether)-iron circulating in the body, the amounts of 58Fe label present in blood samples drawn 14 d after test meal administration were calculated. Calculations followed the principles of isotope dilution and considered that the iron stable isotopes were not monoisotopic (26).

Fecal samples

Pooled fecal material was analyzed in duplicate after mineralization in a microwave digestion system (MLS 1200; MLS GmbH) with concentrated HNO₃ and H₂O₂ (30%). Aqueous spikes of ⁶⁷Zn, ⁵⁶Mg, and ⁴⁲Ca were added (prepared as ZnCl₂, MgCl₂, and CaCl₂ solutions in 0.1 mol HCl/L) to determine the amount of natural zinc, magnesium, and calcium in the sample according to isotope-dilution principles. Aliquots were dried and redissolved in 6 mol HCl/L for chromatographic separation of copper and zinc from the matrix. The elemental copper concentration in the fecal material was measured by atomic absorption spectrometry (SpectrAA 400; Varian, Mulgrave, Australia) because copper has only 2 stable isotopes and therefore isotope dilution analysis is not an option. Anion-exchange chromatography (AG-I×8, 200–400 mesh; Bio-Rad, Glattbrugg, Switzerland) was used to separate copper and zinc from the matrix by using similar techniques as described earlier (18, 27). The first fraction was kept for further separation of magnesium and calcium. Contamination was monitored during copper separation by the processing of a known amount of pure ⁶⁵Cu spike in parallel to each batch of samples. The second isotopic label (⁶⁷Zn) added to each sample before sample digestion was used to monitor zinc separation blanks.

The fraction containing magnesium and calcium was evaporated to dryness and re-dissolved in 0.7 mol HCl/L. Cation-exchange chromatography (500W X-8, 200–400 mesh; BioRad) was used to separate magnesium and calcium (27). Separation blanks were monitored by using isotopic labels (⁵⁶Mg and ⁴²Ca) added to each sample before digestion. Zinc, magnesium, and calcium fractions were evaporated to dryness under sub-boiling conditions and were stored in polyethylene vials until analyzed. Copper fractions were evaporated to dryness in capped quartz vessels at 90 °C and were heated at 450 °C for 4 h in a muffle furnace (M110; Heraeus Instruments, Hanau, Germany) to destroy organic matter that might interfere with the isotope ratio measurements. Isotopic ratios of zinc, magnesium, and calcium were determined for each element by TIMS based on the generation of Zn⁺, Mg⁺, and Ca⁺ ions in a rhodium double-filament ion source similar to a previously described technique (27, 28). A TIMS measurement technique using Cu(CN)₂⁻ ions was developed for copper isotopic ratio measurements to improve the precision of isotopic analysis (T Walczyk, personal communication, 2000). Samples (5–10 μg Cu) were loaded as CuCl₂ in aqueous solution together with 40 μg Zn as ZnCl₂ and 100 μg NaCN on top of the evaporation filament. The solution was dried electrothermally at 0.8 A while the ionization filament remained unloaded. Measurements were performed at ionization filament temperatures of 930 °C and evaporation filament temperatures of 350 °C. Ion intensities for the main signal were on the order of 1–2 × 10⁻¹¹ A.

The American Journal of Clinical Nutrition
was on the order of 0.05% for the $^{65}$Cu,$^{63}$Cu isotopic ratio, 0.1–0.3% for the $^{60}$Zn,$^{62}$Zn and $^{70}$Zn,$^{64}$Zn isotopic ratios, 0.2–0.4% for the $^{25}$Mg,$^{27}$Mg and $^{46}$Mg,$^{48}$Mg isotopic ratios, and 0.2–0.3% for the $^{42}$Ca,$^{43}$Ca and $^{44}$Ca,$^{45}$Ca isotopic ratios, respectively. The shift in isotopic ratios in the fecal material was in the range of 10–20% for copper and 30–50% for zinc, 12–20% for magnesium, and 5–15% for calcium compared with the natural isotopic ratios.

Calculation of fractional apparent absorption

The apparent absorption of zinc, copper, magnesium, and calcium was calculated based on the excreted amounts of the $^{70}$Zn, $^{65}$Cu, $^{25}$Mg, and $^{44}$Ca isotopic labels in 72-h fecal pools according to previously described principles (29). Absorbed isotopic labels were determined by subtracting the amount of isotopic labels found in the fecal pools from the administered doses. The total amount of isotopic labels in the fecal pools was calculated from measured isotopic ratios and measured total element amounts. Calculations were based on isotope dilution principles and considered that the isotopic labels were not mono-isotopic (26). Data are presented as fractional absorption of the administered dose. All calculations were performed by using in-house designed software (including control routines) for commercially available spreadsheet software (EXCEL 97, MICROSOFT OFFICE, WINDOWS NT; Microsoft Corporation, Redmond, WA).

Food analyses

Samples of infant cereals were mineralized by microwave digestion in a HNO$_3$/H$_2$O$_2$ mixture (MLS 1200; MLS GmbH) and were analyzed for iron, copper, magnesium, and calcium by electrothermal-flame atomic absorption spectroscopy (SpectraAA 400) by a standard addition technique to minimize matrix effects. Phytic acid content was determined by a HPLC technique (30, 31).

Statistical evaluation

Paired t tests (EXCEL 97, MICROSOFT OFFICE, WINDOWS NT; Microsoft Corporation) were used to evaluate differences in erythrocyte incorporation of iron and the apparent absorption of zinc, copper, calcium, and magnesium from test meals fortified with ferrous sulfate and NaFeEDTA. P values <0.05 are referred to as significantly different. Data on erythrocyte incorporation were logarithmically transformed before statistical analysis, and the results are presented as geometric means ±1 SD, −1 SD. All other results are presented as arithmetic means ± SDs.

RESULTS

The infant cereal contained 2.0 ± 0.02 mg Fe, 1.1 ± 0.01 mg Zn, 358 ± 4 mg Cu, 55.6 ± 0.9 mg Mg, and 45.0 ± 0.3 mg Ca per 100 g cereal product before the addition of the stable-isotope labels. Corresponding values for the commercial product used during the prefeeding period were 15.1 ± 0.2 mg Fe, 7.3 ± 0.1 mg Zn, 353 ± 6 mg Cu, 45.0 ± 1.0 mg Mg, and 250.6 ± 22 mg Ca. Phytic acid content was 0.41 g per 100 g.

Two infants were anemic (hemoglobin <110 g/L) and one infant had a low plasma ferritin concentration (<12 μg/L). No significant difference in erythrocyte incorporation of iron stable isotopes was found between the 2 iron fortificants ($P = 0.08$). Geometric mean erythrocyte incorporation of iron was 3.7% (6.8, 2.0; NaFeEDTA) and 4.9% (10.5, 2.3; ferrous sulfate plus ascorbic acid). Apparent absorption of zinc and copper was 21.1 ± 4.7% compared with 20.5 ± 3.9% ($P = 0.77$) and 11.1 ± 6.2% compared with 8.9 ± 3.0% ($P = 0.24$) from the infant cereal fortified with ferrous sulfate and that fortified with NaFeEDTA, respectively. The corresponding values for calcium and magnesium apparent absorption were 50.0 ± 5.5% compared with 50.6 ± 6.9% ($P = 0.82$) and 49.6 ± 7.4% compared with 47.9 ± 6.1% ($P = 0.53$).

DISCUSSION

In the present study, we observed no significant difference in erythrocyte incorporation of iron stable-isotope labels when infants were fed a complementary food based on wheat and soy and fortified with either NaFeEDTA or ferrous sulfate plus ascorbic acid. It is important to stress that ferrous sulfate was evaluated in the presence of ascorbic acid: under these optimal conditions, both iron compounds were equally efficient in providing bioavailable iron from an inhibitory meal.

To our knowledge, the iron bioavailability of NaFeEDTA and ferrous sulfate plus ascorbic acid has not been directly compared previously. In one of our earlier studies, we evaluated the enhancing effect of ascorbic acid and Na$_2$EDTA on iron bioavailability from a cereal-based Peruvian school breakfast meal fortified with ferrous sulfate (14). After the addition of either ascorbic acid or Na$_2$EDTA at molar ratios of 0.6–0.7 relative to iron, no significant difference in iron bioavailability was observed between test meals (14). Thus, both ascorbic acid and Na$_2$EDTA are equally efficient in enhancing iron bioavailability from ferrous sulfate in a Peruvian school breakfast meal. Several studies have reported on the potent enhancing effect of ascorbic acid on iron bioavailability from ferrous sulfate in infants and schoolchildren (4, 9–10, 14). Although the enhancing effect of Na$_2$EDTA on iron bioavailability from inhibitory meals fortified with ferrous sulfate has been shown repeatedly in adults (11–13), there are no other studies in children apart from our study in Peru (14).

The potential usefulness of NaFeEDTA as a fortificant in foods with a high phytic acid content has been shown in several human studies. High relative iron bioavailability from NaFeEDTA added to inhibitory meals, as compared with ferrous sulfate without added ascorbic acid, was shown by MacPhail et al (32), Viteri et al (33), Layrisse & Martinez-Torres (34), Martinez-Torres et al (35), and Davidson et al (15). However, in less inhibitory meals, iron bioavailability from NaFeEDTA was not significantly different from the iron bioavailability of ferrous sulfate (12, 13, 36). Clearly, the development of a food fortification strategy and, in particular, the selection of an approach to optimize iron bioavailability from the fortified food need careful consideration of the specific conditions relevant to the food fortification vehicle and the target population group. For example, the potential usefulness of NaFeEDTA as a food fortificant for condiments is indicated by the encouraging results from earlier efficacy studies (37–39). There has also been renewed interest in the use of NaFeEDTA as a food fortificant, in particular for iron fortification of liquid condiments, such as fish sauce and soy sauce, because NaFeEDTA can be added without provoking unacceptable organoleptic changes (reviewed by Fidler et al, 36). In addition, a recent efficacy study in Vietnam provided...
convincing data that fortified fish sauce would have a significant positive effect on the iron status of anemic Vietnamese women (40).

The use of NaFeEDTA as a food fortificant, however, is limited to supervised food fortification programs that provide no more than 0.2 mg Fe·d⁻¹·kg body wt⁻¹ (41). For infants, the daily intake of iron provided by NaFeEDTA-fortified foods would therefore be limited, and NaFeEDTA is not currently used to fortify industrially produced complementary foods. During the present study, the fortification level was according to that in similar commercial infant cereals (10 mg Fe/100 g dry cereal), and the 2 servings of infant cereal consumed per day provided 4 mg Fe. However, because the infants participating in the present study weighed 5.7–8.0 kg, only 1.2–1.6 mg Fe/d could be provided by NaFeEDTA in order to not exceed the limit of 0.2 mg Fe/kg body wt set by the Joint FAO/WHO Expert Committee on Food Additives (41).

A major reason for the reluctance to use NaFeEDTA in food fortification programs is related to concern over the possible negative influence of NaFeEDTA on the metabolism of other essential nutrients because EDTA is a strong metal chelator. After digestion, a small fraction (≈5%) is absorbed and excreted in urine, whereas the majority is lost via the gastrointestinal tract (42). The absorbed EDTA moiety could negatively affect the metabolism of minerals and trace elements by increased urinary excretion. Only limited information is available on the influence of NaFeEDTA on the absorption and excretion of other nutritionally important minerals and trace elements. We reported on the effect of NaFeEDTA (and of increasing levels of Na₂EDTA) on zinc, copper, and calcium metabolism in rats fed zinc-deficient diets based on soy (43). Although the urinary excretion of zinc increased significantly with the inclusion of EDTA in the diet, the fractional absorption of zinc also increased. Thus, the overall effect was that fractional zinc retention increased significantly in rats consuming diets containing added EDTA. In a later study, we investigated the effects of NaFeEDTA added to high-extraction wheat flour on the absorption and retention of zinc and calcium in adult women by using stable-isotope techniques (18). The results showed a positive effect on zinc apparent absorption from bread fortified with NaFeEDTA compared with ferrous sulfate: mean absorption increased significantly from 20.9% to 33.5% (P < 0.05). Urinary excretion of ⁷⁰Zn increased significantly (P < 0.001) during the 6-d balance period when NaFeEDTA was used as a food fortificant (0.91 ± 0.34%) compared with when bread fortified with ferrous sulfate was consumed (0.29 ± 0.21%). However, zinc retention from the labeled test meals was not significantly different (18).

Our previous observation that NaFeEDTA used as a food fortificant results in increased zinc apparent absorption from inhibitory diets (18, 43) was not confirmed in the present study. Apparent zinc absorption was 21.1 ± 4.7% compared with 20.5 ± 3.9% (P = 0.77) from the cereal fortified with ferrous sulfate or NaFeEDTA in the study infants. Limited information is available on zinc absorption from cereal-based complementary foods in infants. For comparison, we previously reported the mean apparent zinc absorption from a less inhibitory infant cereal (made of wheat flour and cow milk) to be 33.9% (range: 19.2–63.9%) in infants, based on fecal excretion of ⁷⁰Zn (20). No influence on calcium absorption or urinary excretion was observed in the previous studies (18, 43).

In the present study, we evaluated the apparent absorption of both calcium and magnesium from test meals fortified with either NaFeEDTA or ferrous sulfate plus ascorbic acid. Our results support the previous finding that calcium absorption is not influenced by the presence of EDTA in the diet and provide new information on the lack of effect of EDTA on magnesium apparent absorption. We are not aware of any earlier report on the influence of NaFeEDTA on magnesium absorption in humans.

Our previous animal study showed no statistically significant influence on the absorption, excretion, or retention of copper (43). In the present study, copper absorption was 11.1 ± 6.2% and 8.9 ± 3.0% (P = 0.24) from test meals fortified with ferrous sulfate or NaFeEDTA, respectively. No comparable data on copper absorption from cereal products in infants are available. However, we recently reported zinc and copper apparent fractional absorption, based on a stable-isotope technique, from soy formula in 9 healthy infants. In the soy formula study (44), mean zinc and copper absorption were 16.7% and 31.2%, respectively. After dephytinization, absorption of zinc increased significantly (x: 22.6%; P < 0.03), whereas copper absorption was not significantly influenced (x: 35.0%; P = 0.34).

In conclusion, iron bioavailability from a cereal-based complementary food fortified with either NaFeEDTA or ferrous sulfate plus ascorbic acid was not significantly different in healthy infants. NaFeEDTA did not influence the apparent absorption of zinc, copper, calcium, or magnesium. These results indicate that NaFeEDTA does not provide any nutritional benefit compared with the combination of a highly bioavailable iron compound and ascorbic acid. No information is available on the urinary excretion of minerals and trace elements in infants consuming NaFeEDTA. For practical reasons, it was unfortunately not possible in the present study to investigate the urinary excretion of minerals and trace elements. Although this remains a potentially important topic for further studies, the current recommendation by the Joint FAO/WHO Expert Committee on Food Additives of 0.2 mg Fe as NaFeEDTA·d⁻¹·kg body wt⁻¹ (41) clearly limits the usefulness of this fortificant for infants and children.

We are indebted to all of the study infants and their parents. The excellent care provided by the nursing staff in the Lora N Thomas Metabolism Ward (Department of Pediatrics, University of Iowa, Iowa City) is gratefully acknowledged.

LD designed the study and was responsible for the overall data analysis and the writing of the manuscript. EZ was responsible for the implementation of the study and for data collection. CZ and TW were responsible for the analysis of stable-isotope ratios in biological samples and for food analyses. EZ, CZ, and RH contributed to the preparation of the final manuscript. None of the authors had any conflicts of interest.

REFERENCES

5. Gillooly M, Torrance JD, Bothwell TH, MacPhail AP, Mills W, Mayet...