

## Original Research Article

## Stable Isotopes (Carbon, Nitrogen, Sulfur), Diet, and Anthropometry in Urban Colombian Women: Investigating Socioeconomic Differences

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**Objectives:** We conducted stable isotope and dietary analyses of women from higher and lower socioeconomic status (SES) groups in Cali, Colombia. The objectives were to test between-group differences in stable isotope, dietary, and anthropometric characteristics, and to evaluate relationships between diet and stable isotope values.

**Methods:** Hair samples from 38 women (mean age 33.4) from higher and lower SES groups were analyzed for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values. Dietary intake was assessed via 24-h recalls. Anthropometric variables measured were body mass index, five body circumferences, and six skinfold thicknesses.

**Results:** Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the higher SES group ( $-16.4$  and  $10.3\text{‰}$ ) were significantly greater than those of the lower SES group ( $-17.2$  and  $9.6\text{‰}$ ;  $P < 0.01$ ), but mean  $\delta^{34}\text{S}$  values did not differ significantly between groups (higher SES:  $4.6\text{‰}$ ; lower SES:  $5.1\text{‰}$ ). The higher SES group consumed a greater percentage of protein than the lower SES group (14% vs. 12% of energy;  $P = 0.03$ ), but the groups did not differ in other dietary characteristics or in anthropometric characteristics.  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values were not correlated with intake of the dietary items predicted (sugars, animal-source protein, and marine foods, respectively). The lower SES group was more variable in all three stable isotope values ( $P < 0.05$ ), mirroring a trend toward greater dietary variability in this group.

**Conclusions:** Stable isotope values revealed a difference between SES groups that was not explained by the dietary data. The relationship between diet and stable isotope composition is complex. *Am. J. Hum. Biol.* 00:000–000, 2014.

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The accurate characterization and quantification of human dietary intake is of fundamental importance to the work of human biologists and nutritional anthropologists. Methods for assessing diets at numerous levels of analysis are well-described, and a number of best-practices methodological reviews are commonly used. Some approaches are geared toward nutrition researchers in general (e.g., Gibson, 2005; Marr, 1971; Rutishauser and Black, 2002), while others are specific to an anthropological audience (e.g., Quandt and Ritenbaugh, 1986). Additionally, for anthropologists a primary goal of dietary assessment is often the identification of the patterns of food consumption, rather than the intake of isolated nutrients only. The various dietary assessment methods employed by anthropologists are alike in that each method is characterized by particular strengths and weaknesses that make it useful and valid in some research contexts, but less appropriate in other contexts. There is a complex tradeoff among accuracy, precision, cost, subject and researcher burden, and resolution with no single method achieving the ideal in all of these characteristics.

Stable isotope analyses are a potential method of dietary assessment that has seen widespread application in biological anthropology. This method relies on the fact that “you are what you eat” (West et al., 2006) and that, for example,  $\text{C}_3$  and  $\text{C}_4$  photosynthetic pathway plants exhibit distinct differences in abundance of the rare stable isotope  $^{13}\text{C}$  (Ehleringer et al., 1997; Farquhar et al., 1989). Thus, the ratio of  $^{13}\text{C}$  to the common stable isotope  $^{12}\text{C}$  within a plant’s tissues is reflective of a particular aspect of the plant’s physiology (i.e., the photosynthetic pathway). By convention, stable isotope ratios are

expressed as  $\delta$  values relative to an international standard in parts per thousand (per mil or ‰); e.g., a  $\delta^{13}\text{C}$  value indicates the  $^{13}\text{C}/^{12}\text{C}$  ratio of a sample relative to the  $^{13}\text{C}/^{12}\text{C}$  ratio of the international standard. Other elements commonly studied in dietary stable isotope analysis include nitrogen, hydrogen, oxygen, and sulfur. The stable isotope ratios of all of these elements reflect distinct aspects of an organism’s diet, physiology, or environment, so multiple elements are often analyzed together (Dawson et al., 2002).

Within anthropology, stable isotope analysis has been used to great effect in understanding early hominin dietary ecology (Lee-Thorp et al., 1994; Richards et al., 2000; Sponheimer et al., 2013), and in reconstructing the diets of archaeological human populations (Ambrose and Krigbaum, 2003; Bogaard and Outram, 2013; Harrison and Katzenberg, 2003; Sealy and van der Merwe, 1986). Additionally, stable isotope analyses have been applied to modern human populations, revealing variability in our food sources (Chesson et al., 2009, 2010, 2011), diet preferences (O’Connell and Hedges, 1999; Valenzuela et al., 2011,

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2012), population geographic origins (Ehleringer et al., 2008; Thompson et al., 2010) and metabolism (Fuller et al., 2005; Hatch et al., 2006). Reitsema (2013) recently provided a broad review of stable isotope applications to the investigation of human physiology, health, and nutrition in both living and archaeological populations.

In one application relevant to modern human dietary patterns, Nardoto et al. (2011) analyzed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the fingernails of 431 subjects living on a rural/urban continuum along the Solimões River in Brazil. Combining the stable isotope values with dietary intake data, the authors found an increase in meat and sugar intake, and a concurrent decrease in fish and manioc flour intake, with increasing size of urban centers in this region. Nardoto et al. (2011) interpret these results as evidence of a nutrition transition associated with increased urbanization in the Brazilian Amazon.

In another recent application, Nash et al. (2012) analyzed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in red blood cells of 231 indigenous subjects in Southwest Alaska. In conjunction with dietary intake data, the authors found that  $\delta^{15}\text{N}$  values were correlated with fish and marine mammal intake, while  $\delta^{13}\text{C}$  values were correlated with intake of market foods made from maize and sugar cane ( $\text{C}_4$  plants), as well as with total market food intake. Nash et al. (2012) concluded that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as measured in red blood cells can serve as biomarkers of dietary change and market integration in indigenous circumpolar populations.

In this study, we analyzed stable isotope ratios of carbon, nitrogen, and sulfur in bulk hair samples of women from Cali, Colombia. Carbon isotope ratios reveal the proportion of  $\text{C}_3$  plants (e.g., rice, wheat) to  $\text{C}_4$  plants (e.g., maize, sugarcane) in the diet (Macko et al., 1999; Schwarcz and Schoeninger, 1991). Equivalently, carbon isotope ratios can also reflect the proportion of  $\text{C}_3$  to  $\text{C}_4$  plant foods in the diets of the domestic animals consumed by human subjects (Ayliffe et al., 2004; McCullagh et al., 2005; Nakamura et al., 1982). Nitrogen isotope ratios are typically used to estimate the contribution of animal-source (AS) foods to the diet (Bol and Pflieger, 2002; O'Connell and Hedges, 1999; Petzke et al., 2005a,b). AS foods include not only meat, but also milk, eggs, and other products derived from these foods. Sulfur isotope ratios are associated with the geospatial origin of the diet. That is, the differing sulfur isotope chemistries of soils in different regions are reflected in the foods produced there (Richards et al., 2001; Valenzuela et al., 2011); this effect is particularly evident between coastal and terrestrial regions (Zazzo et al., 2011). Sulfur isotope ratios can also track with the consumption of marine-source foods (Richards et al., 2003; Thompson et al., 2010). Taken together, the stable isotope ratios of these three elements, carbon, nitrogen, and sulfur, can be used to produce a multifaceted dietary profile, albeit a profile described in rather different terms than the more common dietary data from 24-h dietary recalls.

Here, we present dietary intake, anthropometric, and stable isotope data for a sample of women from two socioeconomic status (SES) groups in Cali, Colombia. In a previous anthropometric survey of this study population, Olszowy et al. (2012) found an increase in the prevalence of obesity among lower SES women between 1988–1989 and 2007–2008 (8–17%), but no significant change in obesity prevalence among upper-SES women over the same time period (2–1%). There is, however, little evidence of a

concurrent dietary shift in the lower SES women (Dufour et al., 2014). Whether there has been a shift in diets of upper-SES women is not known. Additionally, Dufour et al. (1997) observed that lower SES Cali women tended to reduce or eliminate their AS food consumption as a coping strategy during periods of financial constraint.

In light of these prior findings from this population, we expected SES differences in both dietary intake and in anthropometric characteristics. Specifically, we expected that the lower SES group would be characterized by lower intake of total protein and of AS foods, and by greater body mass index (BMI) and anthropometric indicators of adiposity (subcutaneous skinfold thicknesses, body circumferences). These expectations are in line with previous observations of diets and obesity trends in several developing-nation contexts. For instance, Vepa (2004) observed a positive relationship between SES and AS food intake in urban India, and Monteiro et al. (2002) observed an emerging trend in adult obesity in the lower-income segment of Brazil from 1975 to 1997.

We asked whether stable isotope values reflect the observed variation in dietary intake and/or anthropometric characteristics, and whether stable isotope analysis reveals any new sources of within- or between-group variability beyond those offered by the more standard methods. We predicted that: (a)  $\delta^{13}\text{C}$  values will track with the intake of sugars, since the primary contribution of  $\text{C}_4$  plants in this diet is likely to be local sugarcane; (b)  $\delta^{15}\text{N}$  values will be positively associated with the intake of animal-source protein; (c)  $\delta^{34}\text{S}$  values will be linked to consumption of marine-source foods.

## METHODS

### *Environment and SES*

Cali is the second largest city in Colombia, with a population exceeding 2.2 million (Reyes et al., 2007). The city is located 1,000 m above sea level, with a mean annual temperature of  $>24^\circ\text{C}$  and  $\sim 1,500$  mm of rainfall per year. Colombia is an economically developing country classified as an upper-middle income country by the World Bank (World Bank, 2009). In 2008 it did, however, have one of the highest rates of income inequality in Latin America as measured by the poverty rate (42%) and the Gini coefficient (57.2), a measure of the income gap between the rich and the poor (World Bank, n.d.).

SES in this study was defined in terms of residential address using the classification system of the city of Cali (Reyes et al., 2007). In this system, each city block is classified into one of six *estratos* (strata) on the basis of the external appearance of the houses, access to municipal services, and the condition of the streets. The particular characteristics of these six *estratos* are described in detail in Olszowy et al. (2012) and are summarized briefly here. *Estratos* one and two included lower-income neighborhoods on the periphery of the city, characterized by narrow streets and small single-story homes of brick or mostly brick. Neighborhoods in *estratos* three and four had relatively larger homes and wider streets. Finally, the higher-income neighborhoods in *estratos* five and six included more luxurious dwellings, such as apartment or condo complexes, or spacious homes surrounded by walls. Residences in the higher-income neighborhoods tended to have full time security guards. For this study, the six *estratos* were compressed into two socioeconomic groups

for analysis: lower SES, *estratos* one, two, and three; higher SES, *estratos* four, five, and six.

### Subjects

The 38 individuals in this study are a cross-sectional sample of non-pregnant non-lactating women 18–44 years of age living in the city of Cali. The subjects were recruited in 2007–08 as part of a larger-scale anthropometric survey, the results of which have been reported previously (Olszowy et al., 2012). Potential subjects were recruited as a convenience sample from seven different sites in the city of Cali: three medical clinics that spanned the socioeconomic range, one government institute, two elite private schools and one expensive health club. The women recruited from the medical clinics were staff and women accompanying patients, and those recruited from the government institute included administrative assistants and women in residence for occupational training. The women recruited from the schools were teachers and parents of the students, and those recruited at the health club were customers and staff. All women were interviewed regarding their current health and reproductive status, and only women who reported themselves as healthy, not under medical treatment or on medication, and not pregnant or lactating were accepted into the study. Informed consent was obtained from all subjects. The study was approved by the Institutional Review Board of the University of Colorado, Boulder, and the Comité de Investigaciones y Ética en Investigación del Centro Médico Imbanaco in Cali.

### Anthropometry

Anthropometric measurements were all taken by the same experienced technician following the recommendation of Lohman et al. (1988), except that the medial calf skinfold was taken with the subject standing. During measurement, subjects wore a hospital dressing gown and no shoes. Body circumferences were measured in duplicate with a flexible steel tape at five sites: mid-upper arm, waist, hip, mid-thigh, and calf. Skinfold thicknesses were measured in triplicate with a Lange skinfold caliper at six sites: triceps, biceps, subscapular, suprailiac, mid-thigh, and medial calf. All circumference and skinfold measurements were taken on the left side of the body and multiple measurements were averaged. Stature (cm) was measured with a Harpenden stadiometer, and weight (kg) with a digital Seca scale that was calibrated daily. BMI was calculated as weight (kg)/height (m<sup>2</sup>). Following the recommendations of the WHO (2000) a BMI < 18.5 was defined as underweight, 18.5–24.9 as normal weight, 25.0–29.9 as overweight, and ≥30.0 as obese.

### Dietary intake

Dietary intake was assessed using 24-h diet recalls, with one recall collected for each subject. The technicians who administered the 24-h recalls were familiar with the local diet. The 24-h recalls were administered following standard protocols and the multiple pass technique (Gibson, 2005). The recalls were followed with a short interview designed to assess the representativeness of the food intake reported, and to determine whether or not the woman was following a special diet of any kind, in either of which case the subject would have been excluded from

the analysis. No subjects were excluded in the present study.

Dietary intake data were analyzed in two ways: first, by frequencies of food items consumed from different food categories, and second, by intake of total kilocalories and macronutrients; macronutrient intakes were further analyzed to determine consumption of sugars, AS protein, AS fat, and marine-source protein. The frequency of consumption was determined by a simple count of the number of times a food appeared in the food record on different eating occasions. For example, two servings of fruit juice at the midday meal (one eating occasion) was counted as one, whereas the same fruit juice consumed at two different meals (two eating occasions) was counted as two.

We aggregated the foods reported in the recalls into food categories on the basis of shared nutritional characteristics and their role in the diet. Those food categories were: sugared beverages, grains and grain products, vegetables and fruits, animal source foods, soups and stews, and sweets and candies with an additional category for miscellaneous foods. These seven main food categories were further broken down into specific types of foods; for example, the main category of sugared beverages was broken down into five subcategories including fresh fruit juice, coffee and hot chocolate, soft drinks, etc. To analyze caloric intake, a custom-made food composition table was constructed from published food composition tables (INN, 1988; Pennington and Church, 1985; USDA, 1994; USDA, n.d.), proximate analyses of commonly consumed foods, recipes for home-cooked foods, and the nutrient information on packaged food. Overall, the diet is based on rice, bread, and beans, with beef, eggs, milk, and poultry as the main AS foods. There is little indication of seasonal variation in the diet. For example, Dufour et al. (1997) collected three rounds of dietary intake data at 0, 3, and 6 months from 85 low-SES Cali women, and the data revealed no significant differences in the intake of total energy, protein, carbohydrate, or fat between the measurement rounds.

### Stable isotope analysis

Bulk hair samples were collected at the same time as the 24-h diet recalls were administered. Samples were taken by cutting four to five hairs from each subject at the nape of the neck about 2.5–5 cm from the scalp. The hair samples were analyzed for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values at the Stable Isotope Ratio Facility for Environmental Research (SIRFER), University of Utah. Hair samples were washed twice in a 2:1 chloroform/methanol solvent mixture to remove lipids, surface dirt, hair dye, and other contaminants. After air-drying, the samples were subsequently ground into a homogenous fine powder using a ball mill (Retsch; Haan, Germany). Several bulk samples contained too little hair for effective mill grinding; these samples were instead frozen with liquid N<sub>2</sub> and ground by hand with mortar and pestle.

For  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses, 500  $\mu\text{g}$  ( $\pm 10\%$ ) of hair was loaded into tin capsules; for  $\delta^{34}\text{S}$  analysis, 600  $\mu\text{g}$  ( $\pm 10\%$ ) of hair was loaded into tin capsules. Hair samples were analyzed using an isotope ratio mass spectrometer (Finnigan-MAT Delta S; Bremen, Germany) operated in continuous flow mode. Analyses of hair samples for combined carbon and nitrogen as well as for sulfur isotopes were

TABLE 1. Comparison (*t* test) of age and anthropometric characteristics (mean  $\pm$  SD) between lower SES (*n* = 19) and higher SES (*n* = 19) groups

	Lower SES	Higher SES	<i>t</i> ( <i>df</i> = 36)	<i>P</i> value
Age (years)	32.9 $\pm$ 8.0	33.8 $\pm$ 8.3	0.32	0.75
BMI (kg m <sup>-2</sup> )	24.1 $\pm$ 3.1	23.4 $\pm$ 2.4	0.71	0.48
<i>Circumferences (cm)</i>				
Waist	74.3 $\pm$ 5.5	73.3 $\pm$ 5.8	0.54	0.60
Hip	97.0 $\pm$ 6.6	97.3 $\pm$ 5.3	0.16	0.88
Mid-upper arm	27.0 $\pm$ 2.3	26.9 $\pm$ 2.3	0.15	0.88
Thigh	49.0 $\pm$ 4.1	49.4 $\pm$ 3.4	0.33	0.75
Calf	34.6 $\pm$ 2.7	35.1 $\pm$ 2.0	0.62	0.54
<i>Skinfolds (mm)</i>				
Triceps	22 $\pm$ 7	24 $\pm$ 6	0.94	0.36
Biceps	17 $\pm$ 6	17 $\pm$ 6	0.16	0.88
Subscapular	23 $\pm$ 7	22 $\pm$ 7	0.56	0.58
Suprailiac	23 $\pm$ 6	22 $\pm$ 8	0.16	0.87
Mid-thigh	36 $\pm$ 9	33 $\pm$ 7	1.18	0.25
Medial calf	19 $\pm$ 7	20 $\pm$ 6	0.55	0.59

conducted using different instruments, but the analytical procedures were similar. Tin capsules were loaded into a zero-blank autosampler (Costech Analytical; Valencia, CA) interfaced with an elemental analyzer (Carlo Erba; Milan, Italy) where they were flash combusted to produce CO<sub>2</sub>, N<sub>2</sub>, and SO<sub>2</sub> for carbon, nitrogen, and sulfur isotope analysis, respectively. Hair samples were analyzed alongside a set of internal laboratory reference materials that had been previously calibrated against international standards: powdered keratin ( $\delta^{13}\text{C} = -24.0\text{‰}$ ;  $\delta^{15}\text{N} = 5.9\text{‰}$ ) for carbon and nitrogen analyses, and silver sulfide ( $\delta^{34}\text{S} = 17.9\text{‰}$ ), zinc sulfide ( $\delta^{34}\text{S} = -31.9\text{‰}$ ), and powdered eider down ( $\delta^{34}\text{S} = 16.7\text{‰}$ ) for sulfur analysis.

Based on long-term measurements of internal laboratory reference materials, the analytical precision ( $1\sigma$ ) for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values was 0.1, 0.2, and 0.4‰, respectively. Stable isotope ratios are reported using the standard  $\delta$ -notation relative to an international standard in units per thousand (‰) as follows:  $\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the molar ratios of the heavy to light isotopes (e.g., <sup>13</sup>C/<sup>12</sup>C) of the sample and standard, respectively.

#### Statistical analysis

Statistical analysis was performed using IBM SPSS 21. Means of anthropometric characteristics, stable isotope values, and dietary intake values were compared between SES groups via *t* tests, after distributions had been confirmed for normality via the K-S test. Pearson correlation coefficients were used to quantify the relationship between stable isotope and dietary intake values. Levene's tests were used to assess between-group differences in variability. Chi-square tests were used to assess between-group differences in the frequencies of women in the four weight categories, and in the frequencies of foods consumed from the seven food categories. Principal components analysis was employed for two purposes. First, it was used to reduce the dimensionality of the anthropometric data. Principal components with eigenvalues >1 were extracted from the set of five body circumferences and from the set of six subcutaneous skinfold thicknesses. Second, principal components analysis was used to aid in the visualization of SES-group clustering in two-dimensional stable isotope space, with two principal components extracted from the three stable isotope variables.

TABLE 2. Frequency (%) of subjects in BMI weight categories by lower SES (*n* = 19) and higher SES (*n* = 19) groups

	Lower SES	Higher SES
Underweight	2 (11)	0
Normal weight	11 (58)	15 (79)
Overweight	5 (26)	4 (21)
Obese	1 (5)	0

Finally, discriminant function analysis was used to assess the ability of anthropometric characteristics, dietary intake data, and stable isotope values to classify individuals into SES groups. Three models were constructed: (1) an anthropometric model including BMI, five body circumferences, and six subcutaneous skinfold thicknesses as predictors; (2) a dietary model including mean daily intake of kilocalories, carbohydrate, protein, fat, and alcohol as predictors; (3) a stable isotope model containing  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values as predictors. These models were compared to determine which set of predictors, if any, could accurately predict SES group membership. All statistical tests were two-sided with null hypotheses rejected at the  $P < 0.05$  level.

## RESULTS

### Anthropometry

Analyses of age and anthropometric characteristics of the 38 women revealed that lower and higher SES groups did not significantly differ in the means of any of these variables (*t* tests,  $P > 0.05$  in each case) (Table 1).

Individuals were also assigned to weight categories according to BMI values, as shown in Table 2. Women in the higher SES group were classified as normal weight or overweight, while the lower SES group additionally included women who were classified as underweight or obese. In the sample as a whole, two women were classified as underweight, 26 as normal weight, nine as overweight, and one as obese.

The two SES groups did not significantly differ in the frequencies of women in the four weight categories (chi-square test,  $\chi^2 = 3.73$ , *df* = 3,  $P = 0.29$ ).

### Dietary intake

Foods were classified in seven main categories plus 21 subcategories, with frequencies of items in food categories consumed by woman per day in lower and higher SES groups reported in Table 3. The two SES groups did not significantly differ in total intake frequencies of the seven main categories (chi-square test,  $\chi^2 = 4.99$ , *df* = 7,  $P = 0.66$ ). In terms of frequency of consumption, the most important main food categories in the diet were sugared beverages, grains and grain products, vegetables and fruits, and animal source foods.

There were no between-group differences in absolute intake of kilocalories or nutrients by SES group (*t* tests,  $P > 0.05$  in each case), but the higher SES group derived a greater percentage of total calories from protein (*t* test,  $P = 0.03$ ) (Table 4).

Also, the lower SES group was more variable in intake of total kilocalories and protein (Levene's test,  $P < 0.05$  in both cases). Figure 1 shows boxplots of intake of sugars (as % of total carbohydrate intake) and AS protein (as % of total protein intake) in lower and higher SES groups;

TABLE 3. Frequencies of items in food categories consumed by woman per day in lower SES (n = 19) and higher SES (n = 19) groups

Food category	Lower SES	Higher SES
Beverages, sugared, total	<b>3.26</b>	<b>2.63</b>
Coffee and hot chocolate	1.26	0.94
Fresh fruit juice <sup>a</sup>	1.21	1.05
Agua de panela <sup>b</sup>	0.16	0.21
Soft drinks, drink mixes, commercial juice drinks	0.47	0.32
Starch thickened drinks <sup>c</sup>	0.16	0.11
Grains and grain products, total	<b>3.00</b>	<b>2.53</b>
Rice <sup>d</sup>	1.05	0.89
Bread, wheat and crackers	1.16	0.89
Bread, maize/cassava	0.47	0.37
Pastries, cookies	0.26	0.26
Snack foods like savory chips	0.05	0.11
Vegetables and fruits, total	<b>2.37</b>	<b>2.11</b>
Roots, tubers, plantains	0.89	0.74
Legumes	0.37	0.21
Vegetables, other <sup>e</sup>	0.47	0.79
Fruit, fresh	0.63	0.37
Animal source foods, total	<b>2.53</b>	<b>2.05</b>
Beef and pork <sup>f</sup>	0.95	0.47
Eggs	0.32	0.26
Milk and milk products	0.53	0.84
Poultry	0.42	0.21
Fish	0.11	0.05
Processed meat and canned fish <sup>g</sup>	0.21	0.21
Soups and stews	<b>0.26</b>	<b>0.16</b>
Sweets and candies <sup>h</sup>	<b>0.63</b>	<b>0.32</b>
Miscellaneous foods <sup>i</sup>	<b>0.68</b>	<b>1.11</b>

<sup>a</sup>These drinks were typically made in the home by pureeing whole fruit in a blender with water and sugar.

<sup>b</sup>Traditional sugar water drink made with *panela* (brown loaf sugar), sometimes flavored with lime juice.

<sup>c</sup>Includes *coladas*, *mazamorra*, *champus*. *Coladas* are milk-based, sugared beverages thickened with starch. *Mazamorra* is a milk-based beverage thickened with whole kernel maize. *Champus* is a water-based beverage made with whole kernel (or cracked) maize and a variety of finely chopped fruits.

<sup>d</sup>Includes rice-based dishes.

<sup>e</sup>Includes "green" vegetables like cabbage, green onions, green beans and cucumbers, as well as tomatoes and beets. A small proportion of cooked vegetable dishes also contained meat. These dishes were named for the dominant ingredient; e.g., green beans with beef (*habichuelas con carne*).

<sup>f</sup>Pork was rare in the diet, but is included in the category *carne* (meat). Includes mixed dishes which contained meat or fish as well as vegetables. These dishes were named for the dominant ingredient; e.g., beef with carrots (*carne con zanahoria*) and beef with cabbage (*carne con repollo*).

<sup>g</sup>The processed meat was predominately sausage and bologna (*mortadela*).

<sup>h</sup>Includes desserts like puddings.

<sup>i</sup>Includes condiments, added fats, and alcoholic beverages.

these variables are depicted here because they were expected to have important associations with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

Stable isotope values

Mean stable isotope values in the sample overall for  $\delta^{13}\text{C}$  values were  $-16.8 \pm 0.8\text{‰}$  (range  $-18.5$  to  $-15.4\text{‰}$ ), for  $\delta^{15}\text{N}$  values,  $9.9\text{‰} \pm 0.6\text{‰}$  (range  $8.9$ – $10.8\text{‰}$ ), and for  $\delta^{34}\text{S}$  values,  $4.9\text{‰} \pm 0.8\text{‰}$  (range  $3.1$ – $7.4\text{‰}$ ) (Table 5). While there were no significant between-group differences in  $\delta^{34}\text{S}$  values ( $t$  test,  $P = 0.13$ ), the higher SES group had significantly greater values for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $t$  tests,  $P < 0.01$  in both cases).

All three stable isotope values were more variable in the lower SES group (Levene's tests,  $P < 0.05$  in each case). Boxplots of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values in lower and higher SES groups are shown in Figure 2.

Within the sample as a whole, there was a significant but weak positive correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $r = 0.48$ ,  $P = 0.002$ ), and a significant but weak negative correlation between  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values ( $r = -0.33$ ,  $P = 0.045$ );  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values were not significantly

TABLE 4. Comparison ( $t$  test) of daily dietary intake values (mean  $\pm$  SD) between lower SES (n = 19) and higher SES (n = 19) groups

	Lower SES	Higher SES	$t$ (df = 36)	$P$ value
Energy (kilocalories day <sup>-1</sup> )	2017 $\pm$ 856	1838 $\pm$ 608	0.74	0.46
Carbohydrate (g day <sup>-1</sup> )	308 $\pm$ 128	264 $\pm$ 94	1.20	0.24
Sugars (g day <sup>-1</sup> )	93 $\pm$ 44	72 $\pm$ 30	1.72	0.09
Protein (g day <sup>-1</sup> )	61 $\pm$ 30	64 $\pm$ 18	0.29	0.77
AS <sup>a</sup> protein (g day <sup>-1</sup> )	30 $\pm$ 22	33 $\pm$ 13	0.43	0.67
Fat (g day <sup>-1</sup> )	56 $\pm$ 39	58 $\pm$ 26	0.20	0.84
AS fat (g day <sup>-1</sup> )	24 $\pm$ 22	25 $\pm$ 12	0.01	0.99
Macronutrient intake (%)				
Carbohydrate (% energy)	64 $\pm$ 14	58 $\pm$ 10	1.55	0.13
Sugars (% carbohydrate)	32 $\pm$ 13	29 $\pm$ 12	0.74	0.52
Protein (% energy)	12 $\pm$ 3	14 $\pm$ 4	2.32	0.03
AS protein (% total protein)	47 $\pm$ 19	53 $\pm$ 19	0.99	0.33
Fat (% energy)	24 $\pm$ 10	28 $\pm$ 9	1.45	0.16
AS fat (% total fat)	45 $\pm$ 23	47 $\pm$ 28	0.18	0.86

<sup>a</sup>AS = animal source.

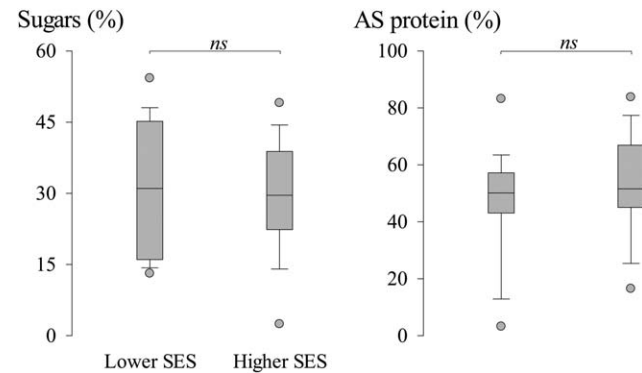


Fig. 1. Boxplots of intake of sugars (% of total carbohydrate) and AS protein (% of total protein) in lower and higher SES groups. Between-group differences not significant for sugar intake ( $t$  test,  $t = 0.65$ ,  $df = 36$ ,  $P = 0.52$ ) or AS protein intake ( $t$  test,  $t = 0.99$ ,  $df = 36$ ,  $P = 0.33$ ). *ns* = not significant. AS = animal source.

correlated. None of the correlations between stable isotope values were significant within the two SES groups.

Within principal components space (82.8% of total variance; Table 6), higher SES individuals clustered more tightly while lower SES individuals displayed more variability, although there was substantial overlap between the two groups (Fig. 3).

Associations between stable isotope values, anthropometric characteristics, and dietary intake data

None of the stable isotope values were significantly correlated with any of the anthropometric characteristics. The 1<sup>st</sup> principal component was extracted from the five body circumference variables (eigenvalue = 3.68, variance explained = 73.6%) and from the six subcutaneous skinfold variables (eigenvalue = 4.37, variance explained = 72.9%); none of the stable isotope values were significantly correlated with these principal components. The correlation between  $\delta^{13}\text{C}$  values and intake of sugars as percentage of total carbohydrate intake was weak and non-significant, both in the sample as a whole ( $r = 0.07$ ,  $P = 0.68$ ), and in the lower SES ( $r = 0.23$ ,  $P = 0.35$ ) or higher SES ( $r = -0.02$ ,  $P = 0.93$ ) groups individually (Fig. 4).

The correlation between  $\delta^{15}\text{N}$  values and intake of AS protein as percentage of total protein intake was also

weak and non-significant, both in the sample as a whole ( $r = 0.30$ ,  $P = 0.07$ ), and within the lower SES ( $r = 0.33$ ,  $P = 0.17$ ) or higher SES ( $r = 0.15$ ,  $P = 0.55$ ) groups individually (Fig. 5).

$\delta^{34}\text{S}$  values were not related to consumption of marine-source protein as percentage of total protein consumption, but only four individuals consumed marine-source foods at all in this data set.

Discriminant function analyses were conducted to evaluate the ability of stable isotope values, anthropometric characteristics, and dietary intake data to classify individuals into lower SES and higher SES groups. These results are reported in Table 7.

Three models were constructed. The stable isotope model contained  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values as predictors; the anthropometric model included BMI, five body circumferences, and six subcutaneous skinfold thicknesses as predictors; the dietary model included mean daily intake of kilocalories, carbohydrate, protein, fat, and alcohol as predictors. While all three models correctly classified ~76–82% of individuals, only the stable isotope model produced a significant discrimination ( $\chi^2 = 15.93$ ,  $df = 3$ ,  $P < 0.01$ ).

## DISCUSSION

In this study, we evaluated dietary intake, anthropometric, and stable isotope data for women from two SES groups in Cali, Colombia. We tested four hypotheses:

- the lower SES group will be characterized by lower intake of total protein and of AS foods, and by greater BMI and anthropometric indicators of adiposity (subcutaneous skinfold thicknesses, body circumferences);

TABLE 5. Mean ( $\pm$  SD) stable isotope values for overall sample ( $n = 38$ ) and by lower SES ( $n = 19$ ) and higher SES ( $n = 19$ ) groups, with comparison ( $t$  test) between groups

	Overall	Lower SES	Higher SES	$t$ ( $df = 36$ )	$P$ value
$\delta^{13}\text{C}$ (‰)	$-16.8 \pm 0.8$	$-17.2 \pm 0.8$	$-16.4 \pm 0.5$	3.30	<0.01
$\delta^{15}\text{N}$ (‰)	$9.9 \pm 0.6$	$9.6 \pm 0.6$	$10.3 \pm 0.4$	4.02	<0.01
$\delta^{34}\text{S}$ (‰)	$4.9 \pm 0.8$	$5.1 \pm 1.1$	$4.6 \pm 0.4$	1.55	0.13

- $\delta^{13}\text{C}$  values will track with the intake of sugars, since the primary contribution of  $\text{C}_4$  plants in this diet is likely to be local sugarcane;
- $\delta^{15}\text{N}$  values will be positively associated with the intake of AS protein;
- $\delta^{34}\text{S}$  values will be correlated with consumption of marine-source foods.

Given the previous observations of this population by Olszowy et al. (2012), we were surprised that in this sample of Cali women, the lower and higher SES groups did not differ in BMI, body circumferences, or subcutaneous skinfold thicknesses. Additionally, the dietary intake values for the two SES groups were very similar, with no between-group differences in absolute daily intake of food energy, macronutrients, or the finer-grained dietary components of sugars, AS protein, and AS fat. Although the higher SES group did derive a greater percentage of total energy from protein, the between-group difference (14% vs. 12%) is unlikely to be of biological significance. This lack of significant SES differences in dietary intake also contrasts with expectations in other developing-nation contexts (e.g., Pelto, 1987; Vepa, 2004), but our observations are consistent with the lack of anthropometric differences in this sample. However, the dietary data were drawn from one 24-h recall, so it is possible that day-to-day dietary variability, and therefore a potential between-group difference, was somewhat obscured in these data.

On the other hand, the SES groups did significantly differ in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, with the higher SES group displaying higher values for both stable isotope ratios. At the same time, the lower SES group showed greater variability in  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values. This variability in

TABLE 6. Component matrix for principal components analysis of stable isotope values (total  $n = 38$ )

	1 <sup>st</sup> principal component	2 <sup>nd</sup> principal component
Variance explained	58.3%	24.5%
Coefficients		
$\delta^{13}\text{C}$	0.80	0.35
$\delta^{15}\text{N}$	0.81	0.27
$\delta^{34}\text{S}$	-0.68	0.73

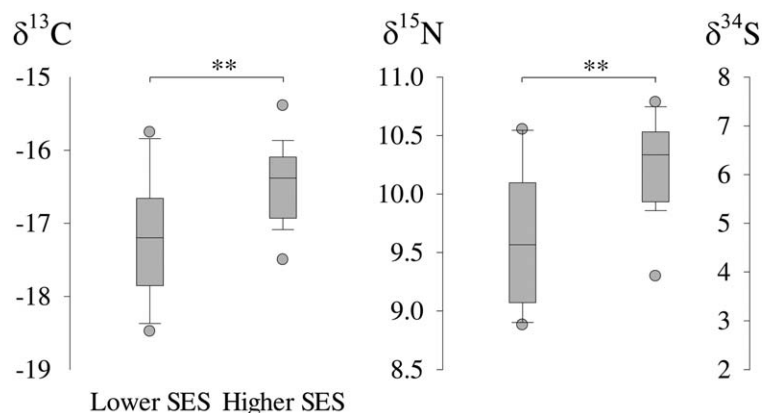


Fig. 2. Boxplots of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values (‰) in lower and higher SES groups. Between-group differences significant for  $\delta^{13}\text{C}$  values ( $t$  test,  $t = 3.30$ ,  $df = 36$ ,  $P < 0.01$ ) and  $\delta^{15}\text{N}$  values ( $t$  test,  $t = 4.02$ ,  $df = 36$ ,  $P < 0.01$ ), but not for  $\delta^{34}\text{S}$  values ( $t$  test,  $t = 1.55$ ,  $df = 36$ ,  $P = 0.13$ ). \*\* =  $P < 0.01$ ; ns = not significant.

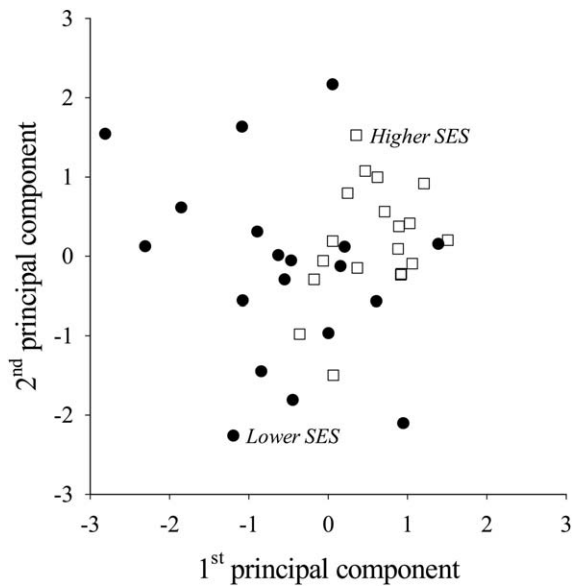


Fig. 3. Lower and higher SES individuals in principal components space. 1<sup>st</sup> and 2<sup>nd</sup> principal components extracted from  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values. Filled circles = lower SES; open squares = higher SES.

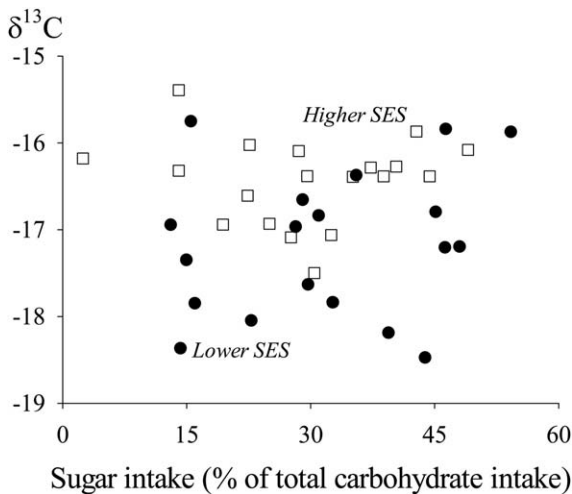


Fig. 4. Relationship between sugar intake and  $\delta^{13}\text{C}$  values ( $\text{‰}$ ) ( $r = 0.07$ ,  $P = 0.68$ ). Filled circles = lower SES; open squares = higher SES.

stable isotope values appears to reflect a trend toward greater dietary variability in the lower SES group. However, the between-group differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are more difficult to explain, due to the lack of corresponding differences in dietary and anthropometric variables.

$\delta^{13}\text{C}$  values in the Cali samples averaged  $-16.8\text{‰} \pm 0.8\text{‰}$ , which is identical to the observations by Valenzuela et al. (2011), who reported a mean value of  $-16.8\text{‰} \pm 0.8\text{‰}$  for hair samples collected in 73 cities across the United States. In contrast, the mean hair  $\delta^{13}\text{C}$  values of 13 Western European countries averaged  $-20.3\text{‰} \pm 0.8\text{‰}$ , indicating a greater contribution of  $\text{C}_3$

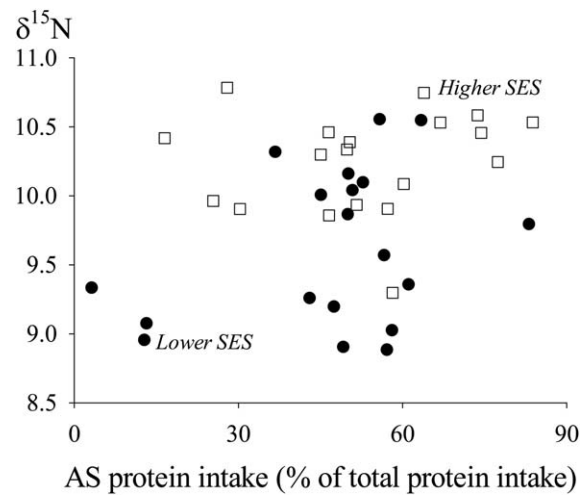


Fig. 5. Relationship between AS protein intake and  $\delta^{15}\text{N}$  values ( $\text{‰}$ ) ( $r = 0.30$ ,  $P = 0.07$ ). Filled circles = lower SES; open squares = higher SES. AS = animal source.

TABLE 7. Discriminant function characteristics (total n = 38)

Characteristic	Variables in the model		
	Stable isotope	Anthropometric	Dietary
% Correct classification	78.9	81.6	76.3
Canonical correlation	0.61	0.59	0.34
Wilks' $\lambda$	0.63	0.66	0.88
$\chi^2$	15.93	12.66	4.17
	( $df = 3$ , $P < 0.01$ )	( $df = 12$ , $P = 0.39$ )	( $df = 5$ , $P = 0.53$ )

Stable isotope variables:  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values. Anthropometric variables: BMI, five body circumferences, and six subcutaneous skinfold thicknesses. Dietary variables: mean daily intake of kilocalories, carbohydrate, protein, fat, and alcohol.

foods to their diets (Valenzuela et al., 2012). Within the Cali sample, the between-SES group difference in mean  $\delta^{13}\text{C}$  values was  $0.8\text{‰}$ . This difference, while statistically significant, is of much lesser magnitude than the difference of  $3.5\text{‰}$  between the Cali and Western European samples.

At the level of the sample as a whole, the similarity between the stable carbon isotope signatures of the United States and Cali potentially reflects a comparable contribution of  $\text{C}_3$  vs.  $\text{C}_4$  plant foods to the diet. Contrary to expectations, however,  $\delta^{13}\text{C}$  values were not linked to the intake of sugars, specifically sugars derived from sugarcane, a principal  $\text{C}_4$ -plant contribution in this diet. Another potential explanation for the stable carbon isotopic similarity between the United States and Cali is a comparable contribution of domestic animals fed on  $\text{C}_3$  vs.  $\text{C}_4$  plants to the diets of these two human populations. For instance, it has been proposed that the relative enrichment of United States  $\delta^{13}\text{C}$  values stems from a preponderance of maize (a  $\text{C}_4$  plant) in the diet, not only as a constituent of processed foods (Jahren and Kraft, 2008) but also as silage for cattle (Bahar et al., 2005; Schmidt et al., 2005). Recently, both Nash et al. (2013) and Fakhouri et al. (2014) demonstrated that the association between  $\delta^{13}\text{C}$  values and dietary sugar consumption was clarified by the inclusion of  $\delta^{15}\text{N}$  values, a marker of AS

protein consumption, as a covariate. In the Cali data, however, the relationship between  $\delta^{13}\text{C}$  values and sugar intake remained nonsignificant (both in the sample as a whole and within each SES group) after controlling for either  $\delta^{15}\text{N}$  values or AS protein consumption.

Additionally, the Food and Agriculture Organization of the United Nations (FAO, n.d.) publishes annual food balance sheets that list the total yearly quantities of food commodities available in nations and groups of nations around the world. These data include the quantities of maize available each year for animal feed, manufactured food, and direct human consumption in Colombia, the United States, and Western Europe. After adjusting for population size, the 2008 data indicate that Colombia had less maize available for animal feed and food manufacture purposes than either the United States or Western Europe (FAO, n.d.). On the other hand, Colombia had over three times as much maize available for direct human consumption in 2008 as either the United States or Western Europe (FAO, n.d.). Thus, if maize is a major dietary driver of  $\delta^{13}\text{C}$  values in the Cali population, then the effect is likely due primarily to direct consumption, rather than animal feed or manufactured foods. However, the extent of direct maize consumption reported in this sample of Cali women was low. Further research is needed to clarify the effect of AS foods on  $\delta^{13}\text{C}$  values in this population, particularly since animal feeding practices (and hence the carbon isotope signatures of AS foods) are likely to vary widely even within nations (Chesson et al., 2008).

$\delta^{15}\text{N}$  values in our sample ranged from 8.9 to 10.8‰, with a mean of  $9.9\text{‰} \pm 0.6\text{‰}$ , while the recently observed mean hair  $\delta^{15}\text{N}$  value for the United States reported by Valenzuela et al. (2011) was  $8.8\text{‰} \pm 0.4\text{‰}$ . The Cali  $\delta^{15}\text{N}$  values were high when compared to those of the United States, and also high relative to those of Western Europe, which displayed a mean of  $9.2\text{‰} \pm 0.5\text{‰}$  and a range of 8.3–10.1‰ (Valenzuela et al., 2012). Within the Cali sample, the between-SES group difference in mean  $\delta^{15}\text{N}$  values was 0.7‰, compared with a difference of 1.1‰ between Cali and the United States and 0.7‰ between Cali and Western Europe. Thus, the difference in mean  $\delta^{15}\text{N}$  values between SES groups is of comparable magnitude to the differences between these populations.

Enriched  $\delta^{15}\text{N}$  values in human hair are often interpreted as evidence of increased AS food consumption. For example, O'Connell and Hedges (1999) analyzed hair  $\delta^{15}\text{N}$  values in a sample of UK vegans (consumers of plant foods exclusively), ovo-lacto-vegetarians (consumers of plant foods, eggs, milk, and dairy products), and omnivores. They reported group means of  $6.9\text{‰} \pm 0.5\text{‰}$ ,  $8.7\text{‰} \pm 0.5\text{‰}$ , and  $8.8\text{‰} \pm 0.6\text{‰}$ , respectively, indicating higher  $\delta^{15}\text{N}$  values in groups that consumed more AS foods. Petzke et al. (2005b) also measured hair  $\delta^{15}\text{N}$  values in German vegans, ovo-lacto-vegetarians, and omnivores, reporting group means of  $6.2\text{‰} \pm 0.4\text{‰}$ ,  $7.7\text{‰} \pm 0.5\text{‰}$ , and  $9.9\text{‰} \pm 0.6\text{‰}$ , respectively, again reflecting a positive association between  $\delta^{15}\text{N}$  values and AS food consumption. The mean  $\delta^{15}\text{N}$  value of the German omnivores,  $9.9\text{‰} \pm 0.6\text{‰}$ , was in fact equal to that of the Cali sample as a whole. For comparison, the proportion of AS protein to total protein intake for the German omnivores was  $0.64 \pm 0.11$  (Petzke et al., 2005b), while that of the Cali sample was  $0.50 \pm 0.19$  (range 0.03–0.84). Taken together, these comparative results seem to sug-

gest a relatively high proportion of AS food consumption in the Cali sample, and relatively higher AS food consumption in the higher SES group compared to the lower SES group. However, both ethnographic observation and the dietary data indicate that the Cali diet is not particularly rich in AS foods (Table 3), the between-group differences in AS food consumption were not significant (Table 4), and the correlation between AS protein intake and  $\delta^{15}\text{N}$  values was not significant in this sample ( $r = 0.30$ ,  $P = 0.07$ ; Fig. 5). Thus, the observed difference in  $\delta^{15}\text{N}$  values between lower and higher SES groups remains difficult to explain.

The analysis of  $\delta^{15}\text{N}$  values is complicated by the fact that the  $\delta^{15}\text{N}$  values of plants can vary substantially by environment (Handley et al., 1999; Amundson et al., 2003). Therefore, the  $\delta^{15}\text{N}$  values of animal tissues, including human hair, can vary between geographic regions due to baseline differences in the stable nitrogen isotopic composition of plant foods, in addition to any differences driven by AS food consumption. For example, Piasentier et al. (2003) found significant differences in the mean  $\delta^{15}\text{N}$  values of lamb muscle samples taken from animals fed on similar diets, but from different countries; e.g., 7.8‰ vs. 3.7‰ for animals fed cereal-based diets in Spain and France, respectively. Conversely, Bahar et al. (2005) observed significant differences in mean  $\delta^{15}\text{N}$  values in muscle samples from Irish cattle fed on grass silage (8.3‰) vs. maize silage (6.6‰), with the differences attributable to the distinct  $\delta^{15}\text{N}$  values of the silages themselves. In neither of these two cases were the animals in question, lambs and cattle, fed AS foods.

The implication of these results for the present study is that the relatively high  $\delta^{15}\text{N}$  values of the Cali sample, when compared with the United States and Western Europe, may not be the result of a higher proportion of AS foods in the Cali diet. Rather, the high  $\delta^{15}\text{N}$  values may reflect the distinct stable nitrogen isotopic composition of the plants at the base of the Cali food chain. Additional stable nitrogen isotope analyses of plants in the Cali region are needed to clarify the association between human hair  $\delta^{15}\text{N}$  values and AS food consumption within this local ecological context.

Another stable isotope method for assessing trophic level (i.e., AS food consumption), not utilized in the current study, involves the fractionation or spacing between  $\delta^{13}\text{C}$  values measured in different tissues within the same individual. Specifically, the spacing between apatite and collagen is known to reflect trophic level, with greater spacing in herbivores and lesser spacing in carnivores (Clementz et al., 2009; Lee-Thorp et al., 1989). Therefore, measuring  $\delta^{13}\text{C}$  values simultaneously in apatite and collagen within individuals provides a way of assessing AS food consumption. Obviously, it is not possible to sample bone apatite or collagen in living humans. On the other hand, in mammals there is a close association between  $\delta^{13}\text{C}$  values in tooth enamel apatite and exhaled breath (Passey et al., 2005); breath  $\delta^{13}\text{C}$  values provide a near-instantaneous measure of isotopic composition, in contrast to the time-integrated signal provided by hair. Additionally, there is a close association between  $\delta^{13}\text{C}$  values in bone collagen and hair keratin (Crowley et al., 2010). This suggests that measuring  $\delta^{13}\text{C}$  values in human breath and hair keratin, and subsequently determining the breath-keratin spacing, could produce data analogous to the apatite-collagen spacing values measured from



hard tissues. Thus, future research in Cali could include noninvasive sampling of breath and hair (keratin)  $\delta^{13}\text{C}$  values to assess AS food consumption, thus potentially avoiding some of the difficulties of the stable nitrogen isotope approach described above.

One additional potential influence on  $\delta^{15}\text{N}$  values is energetic stress. Fuller et al. (2005), in a small sample of pregnant women, found an increase in hair  $\delta^{15}\text{N}$  values concurrent with weight loss and/or restricted weight gain associated with morning sickness. Hatch et al. (2006) also reported an increase in hair  $\delta^{15}\text{N}$  values among female clinical patients diagnosed with anorexia nervosa. In both studies, the cause of the elevated  $\delta^{15}\text{N}$  values was presumably the catabolism of body protein stores in response to negative energy balance. It is possible that the women in the Cali sample may have been affected by energetic stress, particularly in the lower SES group, and this may help to explain their relatively high  $\delta^{15}\text{N}$  values. However, none of the Cali women were pregnant or lactating, only two of them were classified as underweight according to BMI, and there was no association between  $\delta^{15}\text{N}$  values and weight, BMI, body circumferences, or skinfold thicknesses. Additionally, all women identified themselves as healthy and not under medical treatment or on medication. Therefore, it does not seem likely that energetic stress was an important driver of  $\delta^{15}\text{N}$  values in this study.

Finally,  $\delta^{34}\text{S}$  values in the Cali sample ranged from 3.1‰ to 7.4‰, with a mean of  $4.9\text{‰} \pm 0.8\text{‰}$ . For comparison, mean  $\delta^{34}\text{S}$  values have been reported as  $3.4\text{‰} \pm 1.1\text{‰}$  for the United States (Valenzuela et al., 2011), and  $6.8\text{‰} \pm 0.9\text{‰}$  (range 6.6 to 7.5‰) for Western Europe (Valenzuela et al., 2012). Here, the isotopic signature of the Cali sample was again more similar to that of the United States than that of Western Europe. A number of factors may help to explain this.

First, marine-source foods (e.g., fish, shellfish) tend to have higher  $\delta^{34}\text{S}$  values than terrestrial foods (Peterson and Fry, 1987). Second, due to the so-called sea spray effect, protein sources (whether plant- or animal-derived) originating in coastal environments can also display elevated  $\delta^{34}\text{S}$  values relative to terrestrial protein sources (Richards et al., 2003; Wadleigh et al., 1994). Third, there is geographic variability in the sulfur isotopic composition of soils (Peterson and Fry, 1987; Krouse, 1989); therefore, the plant and animal foods produced in different regions may have different sulfur isotopic signatures. Fourth, the isotopic signatures of crops can be impacted by the type(s) of fertilizers used, since different fertilizers exhibit a great range of variability in  $\delta^{34}\text{S}$  values due to differing manufacturing methods and geochemical origins (Vitòria et al., 2004). For these reasons, it is difficult to isolate the key factors explaining why the Cali sample was similar in  $\delta^{34}\text{S}$  values to the United States, but not to Western Europe. However, the data are broadly consistent with a predominantly terrestrial origin of the Cali diet.

More specifically,  $\delta^{34}\text{S}$  values in this sample were not linked to the consumption of marine-source protein as a percentage of total protein intake, but only four individuals in the sample consumed marine-source foods at all. Potentially, a longer-term dietary intake survey would capture more instances of marine-source food consumption, a relatively rare event in this population, and a clearer association with  $\delta^{34}\text{S}$  values might be uncovered.

Overall, the stable isotope data in this study appear to reflect a source of variability that is not apparent in the anthropometric or dietary intake data. There are two general ways of interpreting this situation. The first is that these three sets of measures are actually independent. That is, the stable isotope values do not reflect dietary intake or the processes driving anthropometric variability.

The second interpretation is that the three sets of measures are reflecting dietary variability at different temporal scales. Specifically, the dietary intake data, drawn from one 24-h recall, reflect single days of dietary intake. The stable isotope data, on the other hand, are an aggregate representation of physiological processes occurring on the scale of several weeks preceding the collection of the dietary data. Note that this temporal scale is not inherent to the stable isotope analysis *per se*. Rather, it is a consequence of the physical characteristics of the sample materials analyzed in this study, namely bulk strands of hair several cm in length, which correspond to weeks or months of growth. Finally, the anthropometric data reflect the longer-term outcomes of nutritional status on a scale of weeks to months.

Importantly, a particular diet could be characterized as variable on one temporal scale, yet stable on another. For example, consider a scenario in which low-income individuals must cope with daily food insecurity, but the types of foods potentially available are limited. The composition and nutritional qualities of such a diet could vary greatly from day to day, yet from the standpoint of average dietary intake over the course of a week or a month, the diet could be considered quite stable. This mid-level stability could in turn be nested within a broader-scale pattern of variability, e.g., seasonal variation.

From this perspective, the variability in stable isotope values observed here, in conjunction with the lack of SES-group differences in anthropometric characteristics and short-term dietary intake, is not necessarily contradictory. Instead, the data could be reflecting more-variable and less-variable aspects of the Cali diet when viewed from different temporal scales of analysis. While there is little evidence of temporal patterning in the Cali diet (e.g., seasonality), due to the use of one 24-h recall we are unable to directly assess the representativeness of the dietary intake data on the scale of weeks.

Future research could test this interpretation by assessing dietary and/or stable isotopic variability at several time scales. This could be accomplished by collecting multiple 24-h dietary recalls for each subject over the course of several weeks, for example. Multiple 24-h dietary recalls for each subject would improve the representativeness of the diet for individuals and hence the group (Gibson, 2005). Another possibility would be to tighten the temporal resolution of the stable isotope analyses, i.e., by serially sampling material along individual strands of hair rather than analyzing hair samples in bulk. This could reveal changes in  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values within individuals over time. In any case, widening the temporal breadth of both the dietary intake and stable isotope analyses would allow us to refine our understanding of the variability in the diets of Cali women.

The main limitation of this study is the small sample size, with 38 individuals in total (19 in each SES group). One consequence of this limitation is lowered statistical power. For example, with the sample size of 38, the

correlation between  $\delta^{15}\text{N}$  values and AS protein consumption ( $r = 0.30$ ) was non-significant, but only marginally so ( $P = 0.07$ ). A sample size of 44 would have been sufficient to render this correlation statistically significant ( $P < 0.05$ ). On the other hand, the observed correlation coefficient of  $r = 0.30$  is weak to begin with. In fact, the strongest correlation observed in this study,  $r = 0.48$  ( $P = 0.002$ ) between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, is also weak. However, it can be noted that these correlations are of comparable magnitude to others reported in the literature for associations between stable isotope values and 24-h recall data. For example, in a larger sample ( $n = 230$ ), Nash et al. (2012) report correlations of  $r = 0.52$  between  $\delta^{15}\text{N}$  values and intake of fish and marine mammals, and  $r = 0.46$  between  $\delta^{13}\text{C}$  values and intake of foods made from corn and sugar cane. Additionally, in this study the non-significant between-group differences in anthropometric characteristics did not exceed 1.0 cm for body circumferences or 3 mm for subcutaneous skinfold thicknesses (Table 1), or 6% for macronutrient intake (Table 2). Thus, it does not appear that the limited sample size has obscured between-group differences of potential biological significance.

Another potential consequence of the small sample size is that some finer SES differences may have been obscured. SES in this study was defined according to the *estrato* of a woman's residence. The city of Cali defines six different *estratos*, but for our study, the three lowest *estratos* (one, two, and three) were combined to form the lower SES group, while the three highest *estratos* (four, five, and six) were combined to form the higher SES group. Thus, it is possible that potential differences between extremes of SES, for example between *estratos* one/two and five/six, were attenuated by the inclusion of middle-income groups (i.e., *estratos* three/four).

To address this possibility, we reanalyzed all data with *estratos* three and four excluded. With one exception, none of the reanalyses produced different results than the original analysis, whether in terms of between-SES group differences in dietary, anthropometric, and stable isotope values, or relationships among these variables. The one exception was that in the reanalysis, the SES groups no longer significantly differed in protein intake as a percentage of total energy. It therefore does not seem likely that our original classification of SES groups obscured differences between extremes of SES. However, it must be noted that the sample sizes in the reanalyses (lower SES,  $n = 12$ ; higher SES,  $n = 14$ ) were very small, so the reanalyses had low statistical power. Again, a larger total sample size is needed to fully investigate the fine-grained dietary, anthropometric, and stable isotopic differences among the *estratos* of Cali.

Although limited by small sample size, this analysis underscores the complexity of the relationship between the highly variable diets of living humans and the stable isotope composition of their hair. Despite the lack of clear associations between stable isotope values, dietary intake data, and anthropometric measures in this study, stable isotope analysis did nonetheless reveal new sources of variability that we would not have uncovered using the standard methods of nutritional anthropology alone (i.e., dietary recalls, anthropometry). This indicates that stable isotope analysis can be a valuable tool in the investigation of the diets of living humans. Additionally, the recent clinical applications of stable isotope analysis to detect nutri-

tional stress due to anorexia nervosa/bulimia nervosa (Hatch et al., 2006; Mekota et al., 2006) or pregnancy (Fuller et al., 2005) suggest a potentially important use in nutritional anthropology: they may provide an alternative to anthropometry in revealing negative energy or nutritional stress in free-living populations.

## CONCLUSIONS

In this sample of 38 women living in urban Cali, Colombia, lower and higher SES groups differed only in the intake of protein as a percentage of total energy intake, but not in other aspects of dietary intake, or in anthropometric measures (BMI, five body circumferences, six skinfold thicknesses). The two SES groups also differed in hair  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, but not in  $\delta^{34}\text{S}$  values. Additionally, the lower SES group was more variable in all three stable isotope values, which mirrored a trend toward greater dietary variability in the lower SES group.

Contrary to expectations, neither dietary intake data nor anthropometric measures readily explained the observed variation in stable isotope values. This may be the result of differing temporal scales of the dietary, anthropometric, and stable isotope analyses; the diets of Cali women may be considered variable on certain scales, but not on others. The relationship between diets of living populations and the stable isotope composition of hair is complex. Further research is needed to fully understand the dietary, physiological, and geographic drivers of stable isotopic variability among women in urban Cali, Colombia.

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