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# Field-deployable measurements of free-living individuals to determine energy balance: fuel substrate usage through $\delta^{13}\text{C}$ in breath $\text{CO}_2$ and diet through hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

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## ABSTRACT

Carbon isotopes of breath  $\text{CO}_2$  vary depending on diet and fuel substrate used. This study examined if exercise-induced  $\delta^{13}\text{C}\text{-CO}_2$  changes in substrate utilization were distinguishable from baseline  $\delta^{13}\text{C}\text{-CO}_2$  variations in a population with uncontrolled diet, and compared hair isotope values and food logs to develop an isotope model of diet. Study participants included nine women with diverse Body Mass Index (BMI), age, ancestry, exercise history, and diet. Breath samples were collected prior to and up to 12 h after a 5- or 10 K walk/run. Indirect calorimetry was measured with a smartphone-enabled mobile colorimetric device, and a field-deployable isotope analyzer measured breath  $\delta^{13}\text{C}\text{-CO}_2$  values. Diet was assessed by food logs and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  of hair samples. Post-exercise  $\delta^{13}\text{C}\text{-CO}_2$  values increased by  $0.54 \pm 1.09\text{‰}$  (1 sd,  $n = 9$ ), implying enhanced carbohydrate burning, while early morning  $\delta^{13}\text{C}\text{-CO}_2$  values were lower than daily averages ( $p = 0.0043$ ), indicating lipid burning during overnight fasting. Although diurnal  $\delta^{13}\text{C}\text{-CO}_2$  variation ( $1.90 \pm 0.77\text{‰}$ ) and participant baseline range ( $3.06\text{‰}$ ) exceeded exercise-induced variation, temporal patterns distinguished exercise from dietary isotope effects. Hair  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were consistent with a new dietary isotope model. Notwithstanding the small number of participants, this study introduces a novel combination of techniques to directly monitor energy balance in free-living individuals.

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Breath isotopes; carbon isotopes; energy balance; exercise metabolism; hair isotopes; nitrogen isotopes

## 1. Introduction

Although diet and exercise self-monitoring has been shown to improve lifestyle choices [1–3], poor compliance and accuracy in self-reports for free-living individuals has proven challenging for long-term tracking maintenance in weight control [4,5]. Self-report food logs consistently underreport caloric consumption, and objective validation of self-report is needed for dietary monitoring [1]. While serum or urinary biomarkers of dietary consumption (cholesterol, serum or erythrocyte membrane fatty acids, urinary

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sugars, glycoconjugates) are useful indicators of food consumption [6–10], they represent diet as mediated by metabolism; endogenous production of cholesterol, insulin, and genetic variations moderate biomarkers as a dietary record. Isotopically enriched or natural tracer studies are common in clinical exercise and diet studies [11,12]; however, these studies have been limited to laboratory settings with tightly controlled fuel-switching regimens to maximize the precision on specific macromolecule absorption or utilization and typically involve intensive exercise or dramatic dietary changes [12–14]. With the introduction of field-deployable benchtop cavity ring-down spectroscopy (CRDS) isotope analyzers, breath carbon isotope study conditions can be expanded into more real-world scenarios.

Hair carbon and nitrogen isotopes can validate and reinforce the benefits of dietary self-monitoring. A validated isotope dietary model for standard modern diets could significantly reduce the manual documentation required for average dietary intake tracking. Isotope studies have examined either input (diet) [15–18] or output (fuel substrate usage) [14,19,20] in isolation. However, these techniques have not been tested in a culturally diverse population with no dietary restrictions.

Breath carbon isotopes vary depending on carbon source consumed and fuel substrate burned. Carbon isotopes ( $\delta^{13}\text{C}$ ) of dietary sources vary depending on if carbon was fixed using  $\text{C}_3$  or  $\text{C}_4$  photosynthetic mechanisms.  $\text{C}_4$  plants such as corn and sugar cane have  $\delta^{13}\text{C}$  values of  $-12.4 \pm 4.3\%$ , while  $\text{C}_3$  plants, such as many vegetables and grains, are  $-26.6 \pm 2\%$  [21–23]. Animal protein reflects the animal's dietary sources of carbon [24,25].

Breath carbon isotopes also reflect the metabolic substrate utilized [14,19,20]. Lipids are preferentially enriched in lighter carbon isotopes by approximately 4–5‰ [26,27], much smaller than the potential dietary range of  $\sim 14.2\%$ . Increased lipid burning during activities is reflected by more negative  $\delta^{13}\text{C}\text{-CO}_2$  breath values, while increased carbohydrate oxidation results in less negative  $\delta^{13}\text{C}\text{-CO}_2$  breath values [13,14,28]. The magnitude of the offset is proportional to exercise intensity, although previous studies evaluated exercise after fasting and in laboratory conditions [14,28].

For this study, we hypothesized that changes in carbon isotope composition due to exercise would be smaller than those due to cultural dietary preferences, although they would still be significant compared to an individual's baseline value.

This study was designed to test if changes in breath  $\delta^{13}\text{C}\text{-CO}_2$  value reflect changes in metabolic fuel use due to exercise outside of the laboratory in unrestricted dietary conditions, and to evaluate if these techniques could be applied in studies of free-living individuals during normal daily activities. In addition, we used mobile indirect calorimetry in a smartphone-enabled consumer device to document basal metabolic rate and change with exercise, providing quantitative feedback directly to participants [29–31].

## 2. Materials and methods

Nine female participants volunteered for the study, which required that they: 1) complete an online screening tool, 2) provide baseline measurements of metabolism by indirect calorimetry and  $\delta^{13}\text{C}$  in breath  $\text{CO}_2$ , 3) provide a hair sample for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurement, 4) keep a dietary log for five days, and 5) complete a 5 or 10 km walk or run at their own pace while wearing a heart rate monitor. Exclusion criteria included history of cardiovascular or metabolic conditions that might jeopardize safety during exercise, current

pregnancy, or lactation. All participants completed the PAR-q (Physical Activity Readiness Questionnaire, adapted from American College of Sports Medicine's Health/ Fitness Facility Standards and Guidelines, 1997) and had previously successfully finished a 5 km race. Nine participants were selected from a pool of 28 eligible volunteers to maximize the diversity of the study population in age, BMI, and exercise history. Participants were not asked to alter their diets. The study was approved by Arizona State University's Institutional Review Board under study #00004137.

Prior to and after the race, metabolism measurements using a portable indirect calorimeter (Breezing<sup>®</sup>, Phoenix, AZ) were made and  $\delta^{13}\text{C-CO}_2$  breath samples were collected. Age (range 24–47, mean  $34 \pm 8$ ), BMI values (range 18.5–35.5, mean  $27.3 \pm 6.9$ ), and typical amounts of both aerobic and anaerobic exercise (<1 to >7 h per week) were collected through self-report. Participants completed the exercise at the time and location of their choice.

Breath samples were collected in ALTEF gas sampling bags (Restek Corp., Bellefonte, PA) and analyzed for  $\delta^{13}\text{C}_{\text{VDB}}$  within 48 h of collection by Picarro G2131-I CRDS (Picarro, Sunnyvale, CA) after dilution with  $\text{CO}_2$ -free air. One inch of hair closest to the scalp, approximately ten weeks of growth, was analyzed by elemental analyzer – isotope ratio mass spectrometry (EA-IRMS; Thermo Delta Plus IRMS, Thermo Scientific, Waltham, MA) using standard methods (see Supplemental Information).

Food logs kept by the participants (MyFitnessPal,  $n = 6$ ; handwritten log,  $n = 1$ ; foreign language diet logging software,  $n = 2$ ) were used to estimate caloric intake and proportions of dietary macronutrients. All participants were omnivores, although dietary lifestyle was not a screening factor for recruitment. Component weighting in the isotope diet model was optimized using the food logs and measured  $\delta^{13}\text{C}$  values of homogenized meals described by Whigham et al. [20]. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  dietary composition of the items in the food logs was estimated using common food items reported in the literature (see Supplementary Information). The weighted isotopic value was calculated by:

$$\sum_{i=0}^n (\text{cal}_{\text{food},i} \times \delta^{13}\text{C}_i) / \sum_{i=0}^n \text{cal}_{\text{food},i}$$

The calculations were weighted by grams of carbohydrates, grams of carbohydrates, and grams of sugar, and the results compared. Weighting by the grams of sugar produced poor accuracy in modelled  $\delta^{13}\text{C}$  values when comparing the measured  $\delta^{13}\text{C}$  values of the meals reported by Whigham et al. [20]. Weighting by grams of carbohydrates or calories produced similar accuracy, but weighting by grams of carbohydrates produced a large variability in the offset between measured hair values and modelled diet for participants. An example of the calculations for several meals from Whigham et al. [20] is included in Table 1.

### 3. Results

Metabolic parameters and changes due to exercise are reported in Table 2. Most participants showed increases in their metabolic rate from indirect calorimetry, with increases ranging from + 260 to + 810 kcal/day. There was no significant correlation between metabolic rate and BMI, exercise pace, age, time of day for exercise completion, or basal metabolic rate. There was a moderate negative relationship ( $R^2 = 0.52$ ) between the change in

**Table 1.** Example calculation of modelled  $\delta^{13}\text{C}$  value for diet compared with measured  $\delta^{13}\text{C}$  value<sup>a</sup>.

| Food   | Calories        | Carbohydrates (g) | Fat (g) | Protein (g) | Sugar (g) | $\delta^{13}\text{C}$ , estimate | $\delta^{15}\text{N}$ , estimate |
|--|-----------------|-------------------|---------|-------------|-----------|----------------------------------|----------------------------------|
| Day 1, Breakfast   |                 |                   |         |             |           |                                  |                                  |
| Non-fat milk, 1 cup  | 90              | 13                | 0       | 9           | 12        | -21.40                           | 5.90                             |
| Total cereal, 1.5 cups                                     | 200             | 44                | 1       | 4           | 10        | -19.50                           | 3.20                             |
| Orange juice, 10 oz.                                       | 138             | 33                | 1       | 3           | 28        | -26.00                           | 3.40                             |
| Meal total   | 424             | 90                | 2       | 16          | 50        |                                  | 4.76 <sup>b</sup>                |
| $\delta^{13}\text{C}$ , weighted by calories               |                 |                   |         |             |           | -22.00                           |                                  |
| $\delta^{13}\text{C}$ , weighted by grams of carbohydrates |                 |                   |         |             |           | -22.16                           |                                  |
| $\delta^{13}\text{C}$ , weighted by grams of sugar         |                 |                   |         |             |           | -23.60                           |                                  |
| Measured $\delta^{13}\text{C}$ value <sup>a</sup>          |                 |                   |         |             |           | -22.1                            |                                  |
| Day 1, Lunch   |                 |                   |         |             |           |                                  |                                  |
| Salad, 2.5 cups  | 25 <sup>c</sup> | 5                 | 0       | 2           | 3         | -26.60                           | 3.10                             |
| Newman's Light Balsamic Vinaigrette, 1 Tbl                 | 25              | 2                 | 2       | 0           | 2         | -21.20                           | -                                |
| Low-fat cottage cheese, 4 oz.                              | 90              | 7                 | 3       | 10          | 5         | -21.40                           | 5.10                             |
| Lean Cuisine 3-meat pizza, 1 package                       | 390             | 56                | 9       | 22          | 8         | -23.40                           | 5.40                             |
| Meal total   | 530             | 70                | 14      | 34          | 18        |                                  | 5.18 <sup>b</sup>                |
| $\delta^{13}\text{C}$ , weighted by calories               |                 |                   |         |             |           | -23.11                           |                                  |
| $\delta^{13}\text{C}$ , weighted by grams of carbohydrates |                 |                   |         |             |           | -23.37                           |                                  |
| $\delta^{13}\text{C}$ , weighted by grams of sugar         |                 |                   |         |             |           | -23.13                           |                                  |
| Measured $\delta^{13}\text{C}$ value <sup>a</sup>          |                 |                   |         |             |           | -23.5                            |                                  |
| Day 2, Dinner  |                 |                   |         |             |           |                                  |                                  |
| Lean Cuisine, Chicken with Almonds                         | 270             | 44                | 5       | 13          | 12        | -18.10                           | 4.50                             |
| Non-fat milk, 1 cup  | 90              | 13                | 0       | 9           | 12        | -21.40                           | 5.90                             |
| Salad, 2.5 cups  | 25              | 5                 | 0       | 2           | 3         | -26.60                           | 3.10                             |
| Newman's Light Balsamic Vinaigrette, 1 Tbl                 | 25              | 2                 | 2       | 0           | 2         | -21.20                           | -                                |
| Meal total   | 410             | 64                | 7       | 24          | 29        |                                  | 3.88                             |
| $\delta^{13}\text{C}$ , weighted by calories               |                 |                   |         |             |           | -19.53                           |                                  |
| $\delta^{13}\text{C}$ , weighted by grams of carbohydrates |                 |                   |         |             |           | -19.53                           |                                  |
| $\delta^{13}\text{C}$ , weighted by grams of sugar         |                 |                   |         |             |           | -20.56                           |                                  |
| Measured $\delta^{13}\text{C}$ value <sup>a</sup>          |                 |                   |         |             |           | -19.5                            |                                  |

<sup>a</sup>All meals are described and  $\delta^{13}\text{C}$  measurements are from Whigham et al [20]. Calories, grams of carbohydrate, fat, protein, and sugar are from the MyFitnessPal database.  $\delta^{13}\text{C}$  estimates are taken from values in the literature (see Supplemental Table 1).

<sup>b</sup>Calculated from the average of foods weighted by the grams of protein.

<sup>c</sup>Issues in regards to rounding and accuracy in the MyFitnessPal database are discussed in the Supplemental section.

metabolic rate with reported daily caloric consumption. However, excluding the only two participants with no significant metabolic increase with exercise, who reported consuming <1000 kcal/day, this relationship became weak ( $R^2 = 0.32$ ).

The change in breath  $\delta^{13}\text{C}$ -CO<sub>2</sub> values during exercise was  $+0.54 \pm 1.09\text{‰}$ , indicating an increase in carbohydrate oxidation consistent with the moderate exercise group in McCue et al. [14]. The diurnal breath  $\delta^{13}\text{C}$ -CO<sub>2</sub> range was  $1.90 \pm 0.77\text{‰}$ , also consistent with previous reports [20], while the subjects' baseline variation was much larger at 4.95‰. The first  $\delta^{13}\text{C}$ -CO<sub>2</sub> breath measurement of the day was isotopically lightest ( $p = 0.0043$ ), indicating that participants burned a higher proportion of fat after overnight fasting. The offset between the isotopically modelled diet and hair  $\delta^{13}\text{C}$  value was  $4.04 \pm 1.07\text{‰}$ , consistent with previous reports [32].

The change in breath  $\delta^{13}\text{C}$ -CO<sub>2</sub> values due to exercise was less than the diurnal range for these individuals. Participants' breakfasts were carbohydrate-rich, with 40–100% of the calories coming from carbohydrates prior to running the race. The length of the exercise

**Table 2.** Metabolic and exercise information about participants.

| Participant | Calories /day <sup>a</sup> | Age | BMI <sup>b</sup> | Pace (min/km) <sup>c</sup> | Baseline TEE (kcal/day) <sup>d</sup> | $\Delta$ TEE (kcal/day) <sup>e</sup> | Breath $\delta^{13}\text{C}_{\text{VPDB}}$ (‰) <sup>f</sup> | $\Delta^{13}\text{C}^g$ | Daily range $\delta^{13}\text{C}_{\text{VPDB}}$ (‰) <sup>h</sup> |
|-------------|----------------------------|-----|------------------|----------------------------|--------------------------------------|--------------------------------------|---|-------------------------|--|
| 1           | 1797                       | 27  | 21.6             | 7:01                       | 2000                                 | +260                                 | -21.80  | -0.66                   | 1.66   |
| 2           | 2293                       | 33  | 21.9             | 7:03                       | 2160                                 | +810                                 | -23.21  | 0.93                    | 0.93   |
| 3           | 906                        | 26  | 18.5             | 12:36                      | 1150                                 | +10                                  | -23.12  | 0.55                    | 0.55   |
| 4           | 1019                       | 43  | 35.1             | 12:00                      | 1580                                 | +260                                 | -20.19  | 1.15                    | 2.73   |
| 5           | 1553                       | 35  | 23.3             | 8:00                       | 2320                                 | +330                                 | -22.64  | 0.67                    | 2.18   |
| 6           | 943                        | 37  | 34.1             | 11:34                      | 1710                                 | -210                                 | -22.45  | -1.15                   | 2.65   |
| 7           | 1585                       | 24  | 22.9             | 6:01                       | 2870                                 | +330                                 | -23.25  | 2.61                    | 2.61   |
| 8           | 1057                       | 47  | 32.6             | 13:12                      | 2250                                 | +510                                 | -20.47  | -0.08                   | 1.68   |
| 9           | 1343                       | 38  | 35.5             | 13:12                      | 2110                                 | n/a                                  | -21.10  | 0.81                    | 2.09   |
| average     | 1388                       | 34  | 27.3             |                            | 2017                                 | 288                                  | -22.03  | 0.54                    | 1.90   |
| $\sigma$    | 465                        | 8   | 6.9              |                            | 492                                  | 305                                  | 1.19  | 1.09                    | 0.77   |

<sup>a</sup>Calories per day are the average from the self-reported dietary logs.

<sup>b</sup>BMI is calculated from participants' self-reported height and weight.

<sup>c</sup>Pace is the average speed of the participant during the race.

<sup>d</sup>Baseline TEE (Total Energy Expenditure) is the metabolic rate from the indirect calorimetry at the intake interview.

<sup>e</sup> $\Delta$ TEE is the change in metabolic rate from the indirect calorimetry measurements immediately after the race from immediately prior the race.

<sup>f</sup>Breath  $\delta^{13}\text{C}$  is the isotope composition of the first breath collection of the race day.

<sup>g</sup> $\Delta^{13}\text{C}$  is the change in carbon isotope composition in breath from immediately prior to immediately after the race; positive values suggest an increase in carbohydrate oxidation.

<sup>h</sup>The daily range in  $\delta^{13}\text{C}$  indicates the range of measured breath  $\delta^{13}\text{C}$  values for the day of the race; participants 2 and 3 only provided samples from immediately prior and immediately after the race available, so the listed range is a minimum.

period was insufficient to expend the carbohydrates consumed plus stored glycogen, so participants never switched to predominantly lipid utilization.

$\delta^{13}\text{C}$  hair values and breath  $\delta^{13}\text{C}\text{-CO}_2$  values were correlated ( $R^2 = 0.69$ ), with breath values offset  $-4.33 \pm 0.86\text{‰}$  from hair (Table 3). The observed isotopic difference between diet and hair is expected due to biological mechanisms during dietary absorption and subsequent keratin and lipid production, and is consistent with previous studies [32].

Hair  $\delta^{15}\text{N}$  values were offset  $+4.66 \pm 0.49\text{‰}$  compared to the modelled diet value, and hair and diet values were correlated ( $R^2 = 0.66$ ). This offset between hair and diet is consistent with literature estimates [15]. Although  $\delta^{15}\text{N}$  values are commonly considered a trophic-level indicator, the study's omnivorous subjects still demonstrated a range of  $\sim 2.5\text{‰}$  in  $\delta^{15}\text{N}$  values [16]. From the dietary logs, the proportion of marine foods consumed was more important than the proportion of animal-derived protein consumed, which is also consistent with previous findings [25,33].

## 4. Discussion

### 4.1. Breath $\delta^{13}\text{C}$ values and exercise

Breath carbon isotopes analysis using enriched tracers is a common technique for monitoring fuel usage in exercise studies. Previous studies examined exercise-induced changes in natural breath  $\delta^{13}\text{C}\text{-CO}_2$  values to evaluate the magnitude of the correction to substrate utilization calculations during spiked isotope tracer studies [14]. Naturally occurring  $\delta^{13}\text{C}$  values have also been used in exercise studies to label and track the fate of specific metabolites, but have typically involved depletion of endogenous substrates through intensive exercise, prior to administration of the isotopically labelled fuel substrates [11–13,34].

**Table 3.** Isotopic and dietary information about participants.

| participant | calories /<br>day | % of calories from<br>carbohydrates | % of calories<br>from protein | % of calories<br>from fat | diet $\delta^{13}\text{C}_{\text{VPDB}}$<br>(‰) (model) | breath  | hair                                       | hair $\delta^{13}\text{C}_{\text{VPDB}}$<br>diet $\delta^{13}\text{C}_{\text{VPDB}}$ | diet $\delta^{15}\text{N}_{\text{AIR}}$<br>(‰) (model) | hair   | hair $\delta^{15}\text{N}_{\text{AIR}}$<br>diet $\delta^{15}\text{N}_{\text{AIR}}$ |
|-------------|-------------------|-------------------------------------|-------------------------------|---------------------------|---|---|--|--|--|--------|--|
|             |                   |                                     |                               |                           |   | $\delta^{13}\text{C}_{\text{VPDB}}$<br>(‰) <sup>b</sup> | $\delta^{13}\text{C}_{\text{VPDB}}$<br>(‰) |  |  | carbon |  |
| 1           | 1797              | 49%                                 | 29%                           | 22%                       | -22.08  | -21.80  | -18.18                                     | 3.90   | 6.20   | 10.78  | 4.58   |
| 2           | 2293              | 52%                                 | 25%                           | 23%                       | -24.36  | -23.21  | -18.86                                     | 5.50   | 4.37   | 8.86   | 4.49   |
| 3           | 906               | 57%                                 | 21%                           | 22%                       | -22.42  | -23.12  | -18.98                                     | 3.44   | 3.77   | 8.99   | 5.22   |
| 4           | 1019              | 43%                                 | 29%                           | 28%                       | -21.48  | -20.19  | -16.61                                     | 4.39   | 5.04   | 8.66   | 3.62   |
| 5           | 1553              | 46%                                 | 36%                           | 19%                       | -20.72  | -22.64  | -17.45                                     | 3.55   | 4.80   | 8.72   | 4.45   |
| 6           | 943               | 36%                                 | 32%                           | 32%                       | -21.60  | -22.45  | -17.20                                     | 4.80   | 4.27   | 9.16   | 4.89   |
| 7           | 1585              | 51%                                 | 33%                           | 16%                       | -23.71  | -23.25  | -18.56                                     | 5.44   | 3.23   | 8.30   | 5.07   |
| 8           | 1057              | 47%                                 | 37%                           | 17%                       | -20.40  | -20.47  | -17.61                                     | 2.39   | 4.40   | 9.51   | 5.11   |
| 9           | 1343              | 60%                                 | 28%                           | 12%                       | -18.81  | -21.10  | -15.81                                     | 2.99   | 5.06   | 9.55   | 4.49   |
| average     | 1388              | 49%                                 | 30%                           | 21%                       | -21.73  | -22.03  | -17.70                                     | 4.04   | 4.51   | 9.17   | 4.66   |
| $\sigma$    | 465               | 7%                                  | 5%                            | 6%                        | 1.69  | 1.19  | 1.06                                       | 1.07   | 0.85   | 0.72   | 0.49   |

<sup>a</sup>Calories per day and the percentage of different macronutrients are from the self-reported diet logs. The percentage of macronutrients was normalized to 100%, due to inaccuracies and rounding in the food database (see discussion in supplemental material).

<sup>b</sup>Breath  $\delta^{13}\text{C}$  is the isotope composition of the first breath collection of the race day.

Despite an unrestricted diet and a wide range of baseline  $\delta^{13}\text{C}$  values, the average increase in  $\delta^{13}\text{C}$  values with exercise in the current study was consistent with that seen in the moderate exercise group in highly controlled laboratory studies [14]. This suggests this technique is useful in a wide range of applications representative of average individual's daily lives.

#### 4.2. Hair $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ values and diet

Poor accuracy in dietary self-reporting is well known [1], but hair isotopes can provide objective summaries of weekly or monthly consumption of amount and type of protein and type of carbohydrate. Although widely applied for decades in anthropology [25], this technique of analyzing hair isotopes has received attention in modern dietary studies only relatively recently [15–18,35]. Human diets often vary widely in the proportion of macronutrients, from cultural preferences to dietary choices such as ketogenic, low-fat, or Atkins diet plans. In addition, it is often difficult and expensive to monitor people in controlled settings on restrictive diets and exercise plans for weight loss. Extreme dietary restrictions or intensive exercise may be successful short-term [36], but are not as relevant to the vast majority of first-world peoples with access to a wide variety of food choices. However, examining dietary strategies for successful weight loss and maintenance through non-invasive segmental hair examination may provide useful comparisons about the long-term success of various dietary strategies. Segmental hair isotopic analysis can corroborate food logs kept during dietary modification, while compound-specific isotope ratios may eventually provide more specific biomarker information [23].

#### 4.3. Hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values: Impact of geographic origins and cultural ancestry

BMI in study participants is strongly correlated with hair  $\delta^{13}\text{C}$  values ( $R^2 = 0.78$ ), which may suggest that a heavy corn-based diet is associated with overweight or obesity. However, other studies do not support such an interpretation. Nash et al. [33] showed  $\delta^{13}\text{C}$  in red blood cells was correlated with sugar consumption in a Yup'ik study population ( $n = 48$ ), but there was no correlation between  $\delta^{13}\text{C}$  values and BMI. Our study had significant diversity in cultural ancestry (Asian = 3; Hispanic = 2; non-Hispanic Caucasian = 4). Excluding the three Asian participants in our study, who all consumed a diet similar to their regions of origin, causes the correlation between hair  $\delta^{13}\text{C}$  values and BMI to drop to 0.002. Hence, culture is a stronger predictor of  $\delta^{13}\text{C}$  than BMI, a correlation that is frequently supported in the literature [14,21]. However, the change in breath  $\delta^{13}\text{C}$ -CO<sub>2</sub> values with exercise was seen in all participants with different dietary preferences, as proposed by Whigham et al. [20]. This suggests that as long as baseline variations are considered, the techniques of breath and hair monitoring are robust indicators of dietary change irrespective of initial diet or cultural affiliation.

Breath carbon isotopes indicated a higher proportion of lipids burned in the morning, and exhibit increased carbohydrate oxidation during moderate exercise, despite an uncontrolled diet and significant diurnal variation in breath values. The exercise used in this study is in line with federal guidelines for exercise designed to combat obesity, indicating that these techniques do not require high-intensity exercise or elite athletes to produce a measurable signal.

## 5. Conclusion

Hair  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values reflect diet, while changes in  $\delta^{13}\text{C}\text{-CO}_2$  in breath can reveal metabolic change during exercise. This combination of techniques may encourage the study of exercise-related metabolic changes outside of a laboratory setting in a diverse population and provides an isotopic perspective on energy balance in humans. This is a fundamentally new combination of techniques that is relevant to how modern people live their lives, in terms of diet, culture, and exercise.

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## Disclosure statement

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