INVESTIGATING THE IMPACT OF DIET ON THE STABLE ISOTOPE COMPOSITION OF HUMAN SCALP HAIR AND FINGERNAILS

Gunjan Agrawal and Prasanta Sanyal | Indian Institute of Science Education and Research Kolkata, Mohanpur, India

1. A Palatable Introduction

What we know

- **Stable isotope composition of human tissues** serves as biomarker for dietary analysis.
- Application of stable isotopes is expanding in disease diagnosis and understanding human physiological processes.
- Nitrogen ($\delta^{15}N$) and Carbon ($\delta^{13}C$) stable isotope composition of human tissues **reveal dietary patterns and diet-related disorders**.
- **Magnitude of change in $\delta^{15}N$ and $\delta^{13}C$ values** of human tissues act as an indicator of dietary habits.

The Question

How do dietary changes correlate with $\delta^{15}N$ and $\delta^{13}C$ values of human tissues, when other contributing factors such as environmental and individual influences are controlled?

Why it's important

- To **assess the influence of diet and diet-related disorders** on $\delta^{15}N$ and $\delta^{13}C$ values of human tissues.
- To **enhance the global dataset of $\delta^{15}N$ and $\delta^{13}C$ values of human tissues** in which Asia, at present, contribute only 17-18% despite being home to 60% of the world's population.

EGU General Assembly 2024
Abstract: EGU24-537
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IISER KOLKATA

2. Savory Methodology

Study Site

- Study conducted on **residents of IISER-Kolkata** having a remote residential campus.
- Pandemic and remote location **restricted frequent travel.**
- Residents primarily **depended on campus dining facility.**

Sample Collection

- Scalp hair and Fingernails (67) + Only scalp hair (7) = **74 Study Participants**
- Complete **diet history** from dining facility server.
- **Food items (66)** used in campus kitchen.

Aug 2021 — Diet Information

Sept 2021 — Sample Collection

Experimental Analysis

Nitrogen and Carbon stable isotope analysis of scalp hair, fingernails and food items.

Instruments- Flash 2000 Organic Elemental Analyzer and Delta V Plus IRMS.

Diet Calculations

1. Total food consumed-

$$Af \text{ of Individual X} = \sum_{i}^{n} A_{food\ item} \text{ in food dish}_i^n \times N_{servings} \text{ of food dish}_i^n$$

2. Isotope of diet-

$$I_{diet} \text{ of Individual X} = \frac{(Af1 \times f1) + (Af2 \times f2) + \ldots . (Afn \times fn)}{Af1 + Af2 + \ldots . Afn}$$

3. Percentage of animal protein-

$$P \text{ of Individual X} = \frac{Afa1 + Afa2 + \ldots . Afan}{\text{Total protein consumed}} \times 100\%$$

Af : Total amount of a food item	**P :** % of animal protein in diet
$\mathbf{I_{diet}}$ **:** $\delta^{15}N$ and $\delta^{13}C$ of diet	**Afa :** Total amount of a animal protein in a food item
f : $\delta^{15}N$ and $\delta^{13}C$ of a food item	

3. Delectable Results and Discussions

Lacto-vegetarians vs Omnivores

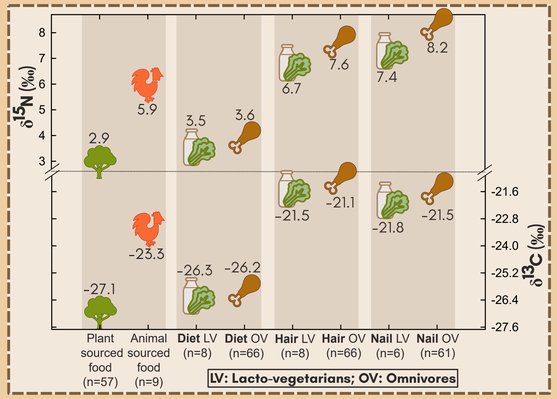

Fig: Mean isotope value of plant and animal-sourced food items, and diet, hair and nail tissue of lacto-vegetarians (LV) and omnivores (OV)

- Isotope value of **diet consumed** by LV and OV is **similar**.
- **Significant difference in mean $\delta^{15}N$ value** between LV and OV **for hair and nail tissue** (T-test).
- **Amount of food intake also influences** isotopic composition of tissues.

Quantity of Animal Protein Intake

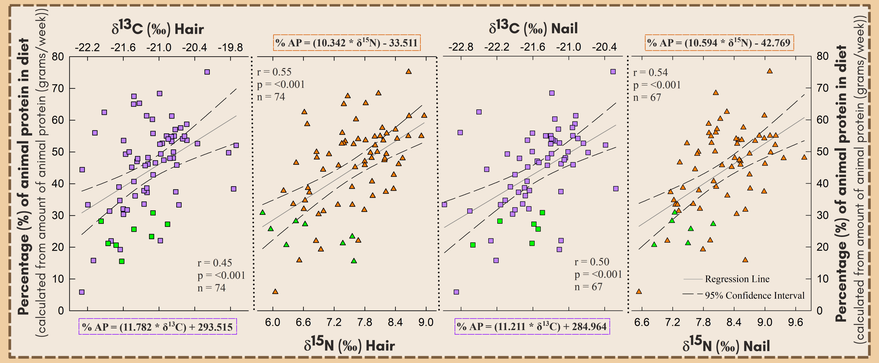

Fig: Bivariate plot between percentage of animal protein in diet and isotopic composition of hair and nail tissue (symbols in green refer to Lacto-vegetarians, while rest are omnivores)

- **Increase in intake** of amount of animal-sourced food and animal protein led to **higher $\delta^{15}N$ and $\delta^{13}C$ values** of human tissues.
- Percentage of **animal protein in diet positively correlated** with $\delta^{15}N$ and $\delta^{13}C$ values of human tissues.

Diet and Health Assessment

Cause	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Reference
5% increase in animal protein intake	↑ 0.4‰ to 0.5‰	↑ 0.4‰ to 0.5‰	This study
Eating disorders	↑ 0.6‰	No change	Hatch et al. 2006
Nutritional stress	↑ 0.54‰	↑ 0.48‰	Neuberger et al. 2013
Liver cirrhosis	↓ 3.2‰	No change	Petzke et al. 2006

Magnitude of change in $\delta^{15}N$ and $\delta^{13}C$ values of human tissues vary with dietary changes and different diet-related disorders.

CONCEPT

Lighter isotopes get eliminated through excretion.

Heavier isotopes are preferentially incorporated in tissues.

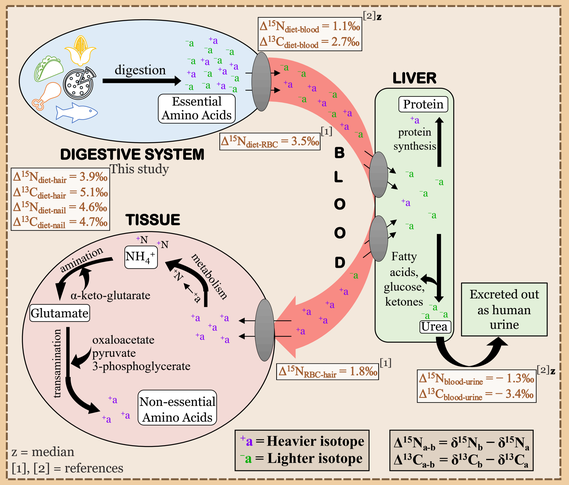

Fig: Metabolic pathway representing enrichment of ^{15}N and ^{13}C in human tissues

4. Sweet Conclusions and Takeaways

$\delta^{15}N$ and $\delta^{13}C$ values of human tissues:

- ✕ Increase with intake of animal-sourced food.
- ✕ Can distinguish between the dietary habits.
- ✕ Can quantify proportion of animal protein in diet.

5. References

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2. Kuhnle et al. 2013 Carbon and nitrogen isotopic ratios of urine and faeces as novel nutritional biomarkers of meat and fish intake
3. O'Connell & Hedges 1999 Investigations into the effect of diet on modern human hair isotopic values
4. Petzke et al. 2005 Choice of dietary protein of vegetarians and omnivores is reflected in their hair protein ^{13}C and ^{15}N abundance

Icons Credit: Microsoft PowerPoint and Noun Project

6. Acknowledgements

Institutional Ethical Committee for approving the study; **UGC, GoI** for PhD scholarship; **SERB-DST, GoI** for instrument; **Study Participants** for informed consent and volunteering

7. Contact

+91 8860927287
/gunjan-ag
gunjan11ag@gmail.com

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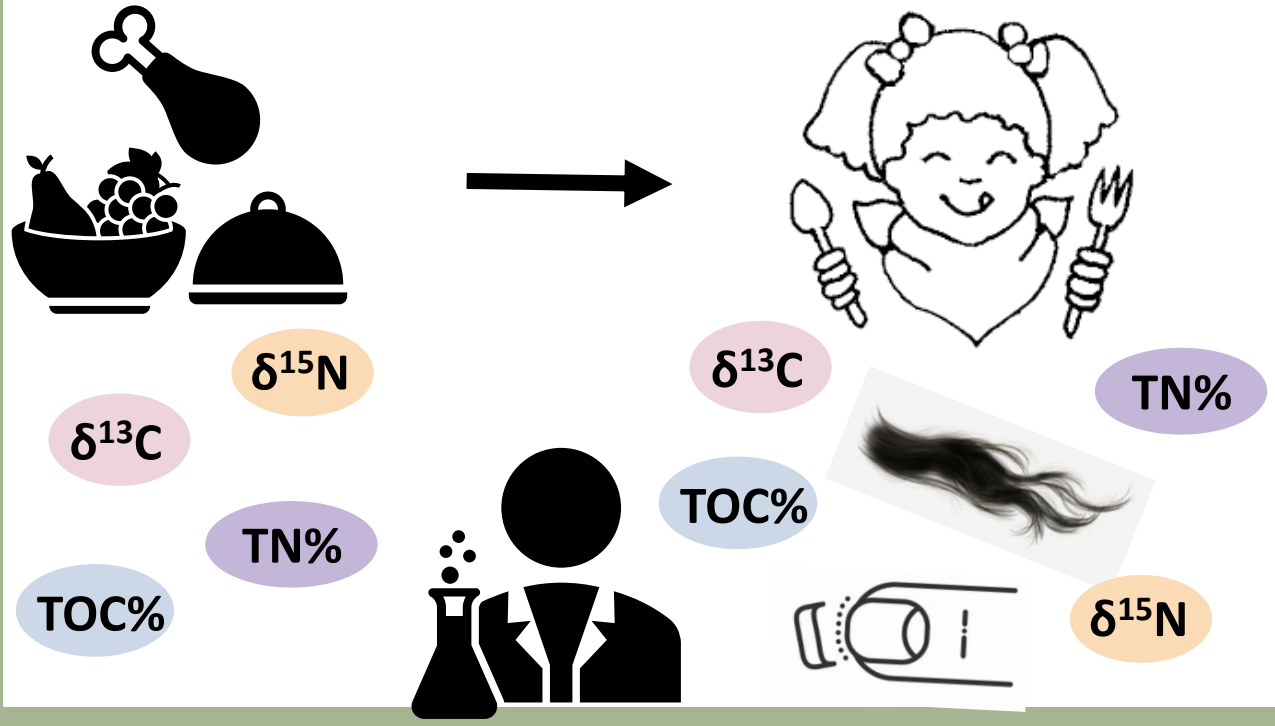
[Poster Presentation Supplementary Materials]

Investigating the impact of diet on the stable isotope composition of human scalp hair and fingernails



Gunjan Agrawal, Prasanta Sanyal

Indian Institute of Science Education and Research Kolkata, Mohanpur, India

Session: BG2.1
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Email: gunjan11ag@gmail.com (GA)
psanyal@iiserkol.ac.in (PS)

HUMAN TISSUE: **Hair and Nails**

Made up of KERATIN -- protein

Reason for the sample choice:

- Non-invasive
- Frequently encountered
- Ease of storage
- No change in chemical composition
- Not easily degraded

BIO-ELEMENTS

Any chemical element that is found in the molecules and compounds that make up a living organism.

Oxygen (65%) > **Carbon** (18.5%) > Hydrogen (9.5%)

> **Nitrogen** (3.2%) > Calcium (1.5%) > Phosphorus (1%)

approx 99% mass of human body

Objective:

1. To distinguish dietary habits based on $\delta^{15}N$ and $\delta^{13}C$ values of human tissues.

2. To determine the relationship between amount of animal-sourced food intake and isotope composition of human tissues.

   In absence of environmental and individual factors

3. To study how two different tissues- scalp hair and fingernails- react with same dietary habit.

Study Site

Study Area: IISER-K Residential campus → Located in remote area

Samples Collected for dietary analysis:

1. Human Scalp Hair – 74
2. Human Finger Nail – 67
3. Food Items – 66

74 Study Participants

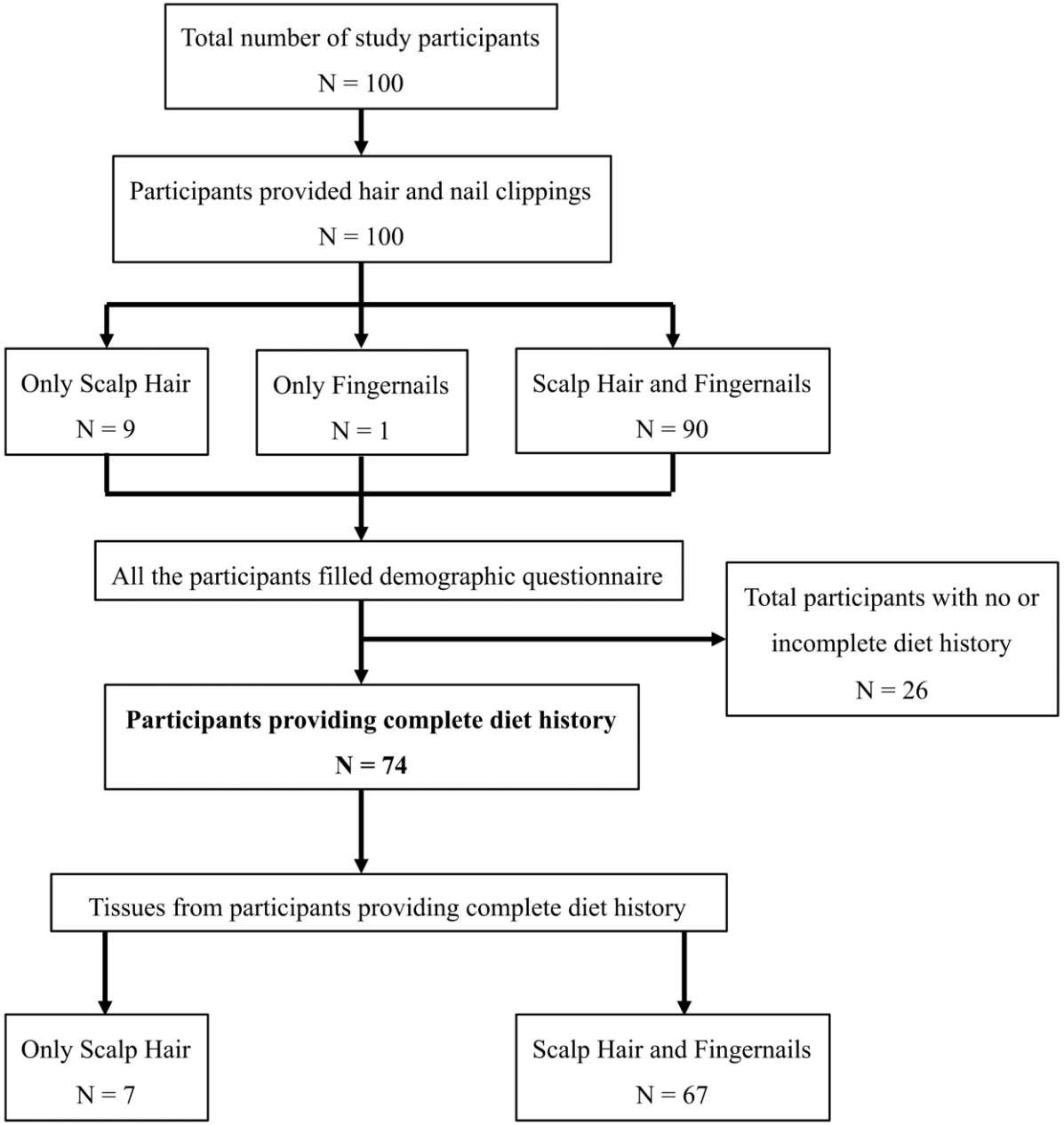
Provided complete diet history

Participant Flowchart

Informed Consent and Ethics Approval Taken

Diet history: Participants provided their 1-week food purchase information.

Food Wastage: Amount of food wastage by participants was considered negligible as a short study to calculate leftover food determined that mean percentage of food wastage by an individual was $2.2 \pm 0.6\%$ of total food consumed.



Methodology

1. **Food Items: 66**
2. **Human Tissues (Hair and Nail): 74 individuals**

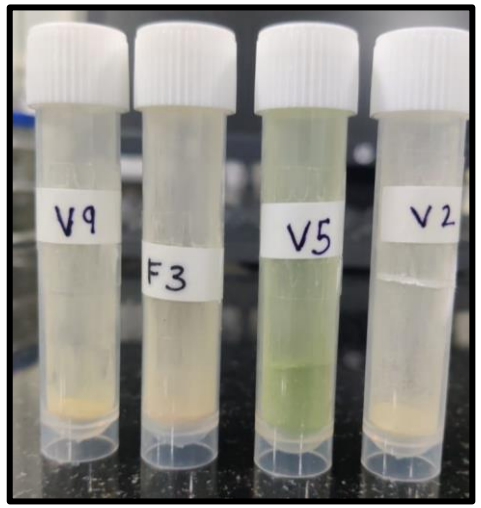
→ Concentration- C_{conc} and N_{conc}
Isotopic Composition- $\delta^{13}C$ and $\delta^{15}N$

Carbon and Nitrogen Concentration → **Calibration Curve**

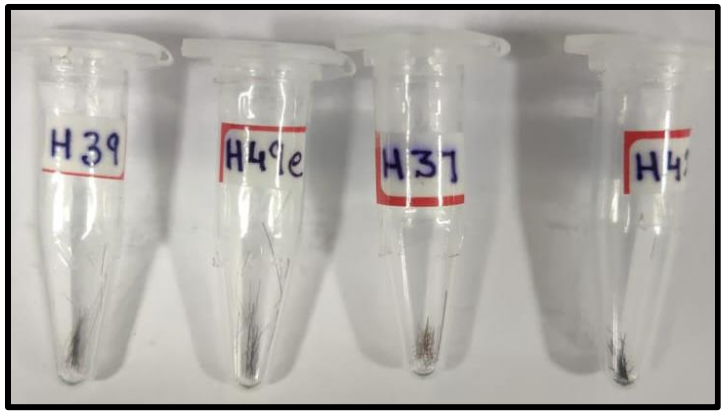
Carbon and Nitrogen Isotopic Composition → **Elemental Analyzer and IRMS**

PREPARED SAMPLES:

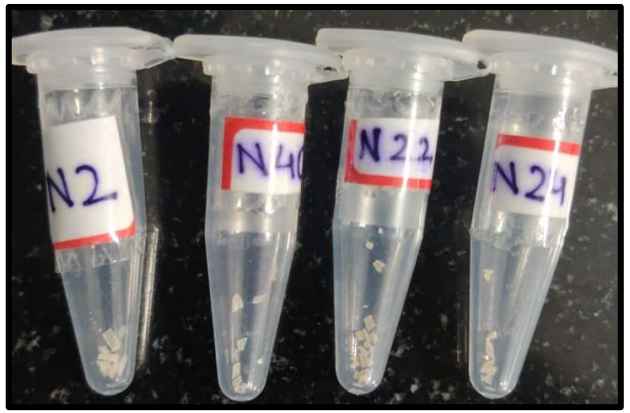
Food Items



Human Hair Tissue



Human Nail Tissue



3. Estimate isotope composition of diet of each individual

Mass-balance Equation →

$$I_{diet,\ x} = \frac{(Af1 * f_1) + (Af2 * f_2) + \ldots\ldots (Afn * f_n)}{Af1 + Af2 + \ldots\ldots Afn}$$

Amount of 1 food item consumed in a week by Individual X

(Obtained from dietary information) (Af)

Concentration and Isotopic composition of food items

(**Obtained from Experimental Analysis**) **(f)**

66 Food Items collected

(Vegetables, cereals, animal meat, fish)

$$Af\ of\ Individual\ X = \sum_i^n (A_{food\ item}\ in\ food\ dish_i^n) \times (N_{servings}\ of\ food\ dish_i^n)$$

Amount of food item in 1 serving of a food dish ($A_{food\ item}$) → 1-week food diary and standard meal plan

Detailed Description of Estimating Diet of an Individual

1. To calculate the amount of food consumed by each individual.

1-week food diary of the individual was obtained from the campus dining facility.

Item	Food item	Amount (gm/serving)
Chana Paneer Masala	Paneer	41.3
	Kabuli chana	48.9
	Onion	30.0
	Tomato	20.0
	Ginger	2.0
	Garlic	2.0
	Green chilli	2.0
	Kaju	2.0
	Melon seeds	2.0

Quantity of a particular food item consumed in a week is calculated.

Sample ID	Items	Quantity (in a week)
98	Chana paneer masala	1.0
	Veg cheese sandwich	1.0
	Roti	15.0
	Milk	4.0
	Egg	4.0

A **standard meal plan**, specific to the campus dining facility, for each dish served in the canteen was created.

Sample ID	Food item	Amount (gms/week) Af
98	Ginger	19.5
	Tomato	220.0
	Potato	457.8
	Onion	320.0
	Garlic	21.0
	Carrot	65.2
	Green chilli	24.5
	Maida	107.2

2. To calculate the concentration/ isotopic composition of diet of each individual.

A

Amount of food consumed by Individual X (**Sample ID 98**)

Sample ID	Food item	Amount (gms/week) Af
98	Ginger	19.5
	Tomato	220.0
	Potato	457.8
	Onion	320.0
	Garlic	21.0
	Carrot	65.2
	Green chilli	24.5
	Maida	107.2

B

Concentration and Isotopic composition of food items (**Obtained from Experimental Analysis**)

Food Item	δ15N f a	δ13C f b	N% f c	C% f d
Green chilli	2.6	-29.5	3.3	63.9
Tomato	4.7	-30.0	2.8	41.3
Ginger	4.4	-30.3	1.0	37.9
Onion	1.1	-28.4	1.2	38.2
Kabuli chana	0.3	-25.7	4.1	45.5

Mass-balance Equation

$$I_{diet,\ x} = \frac{(Af1 * f_1) + (Af2 * f_2) + \ldots\ldots\ (Afn * f_n)}{Af1 + Af2 + \ldots\ldots Afn}$$

3. Estimate amount and percentage of animal protein in diet of each individual

A. Amount of protein consumed by Individual X (***Ap***) in a week:

$$Ap\ of\ Individual\ X = \sum_{i}^{n} [Af\ of\ Individual\ X_i^n \times (P_{food\ item}{}_i^n \div 100)]$$

Amount of 1 food item consumed in a week (**Af**)

Protein content ($\mathbf{P_{food\ item}}$) → Indian Food Composition Table

Had information on amount of protein in 100 gm edible portion of various food items

Similarly, we can calculate amount of animal protein consumed by an individual by inputting only animal-sourced food items in Af.

B. Percentage of animal protein consumed by Individual X (***P***) in a week:

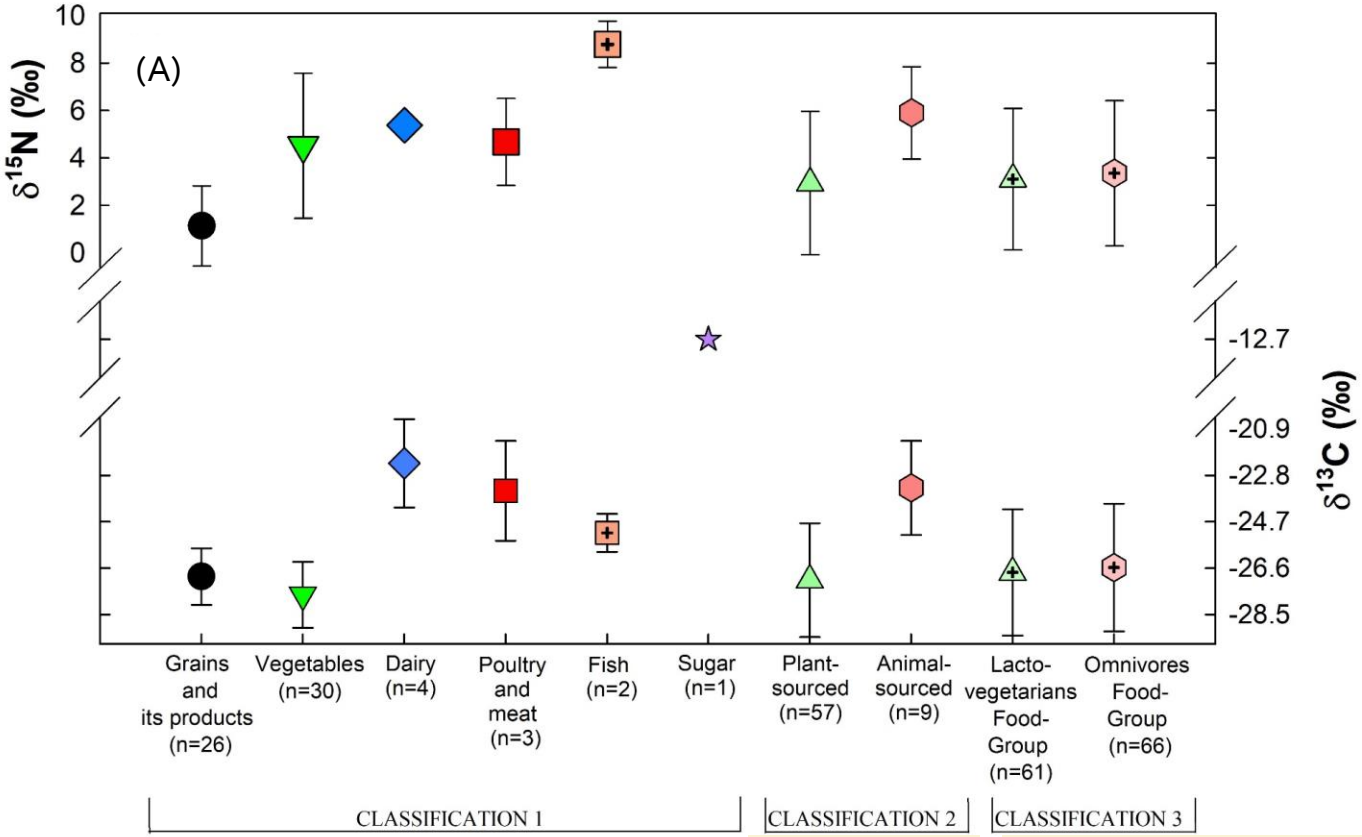
$$P\ of\ Individual\ X = \frac{Apa1 + Apa2 + \ldots.. Apan}{Total} \times 100\%$$

Amount of animal protein consumed in a week (**Apa**)

Total amount of protein consumed in a week (**Total**)

Additional Results: 1)

(A) 66 Food Items (Mean + SD) divided in 3 classifications

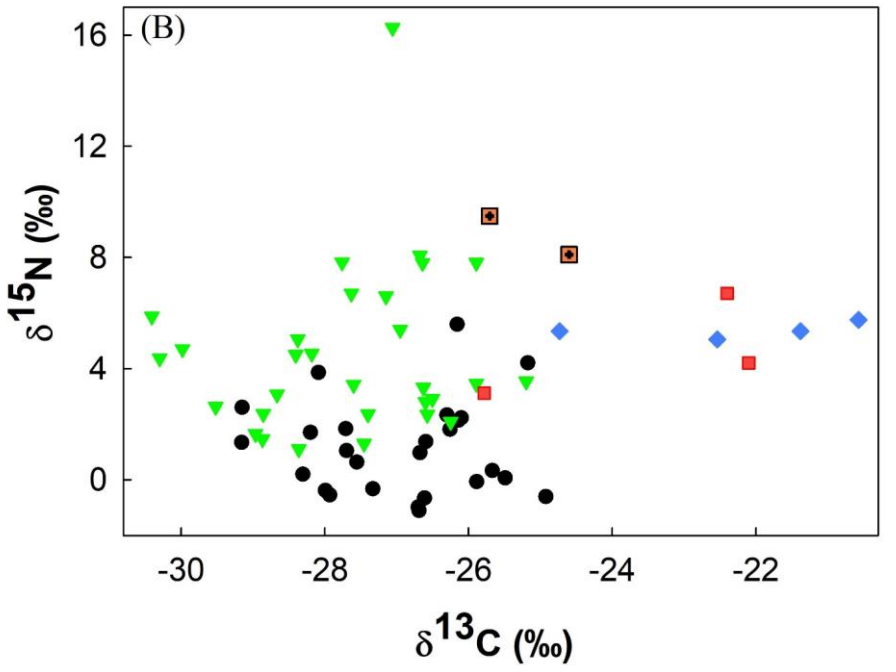


Based on nutritive value

Based on origin

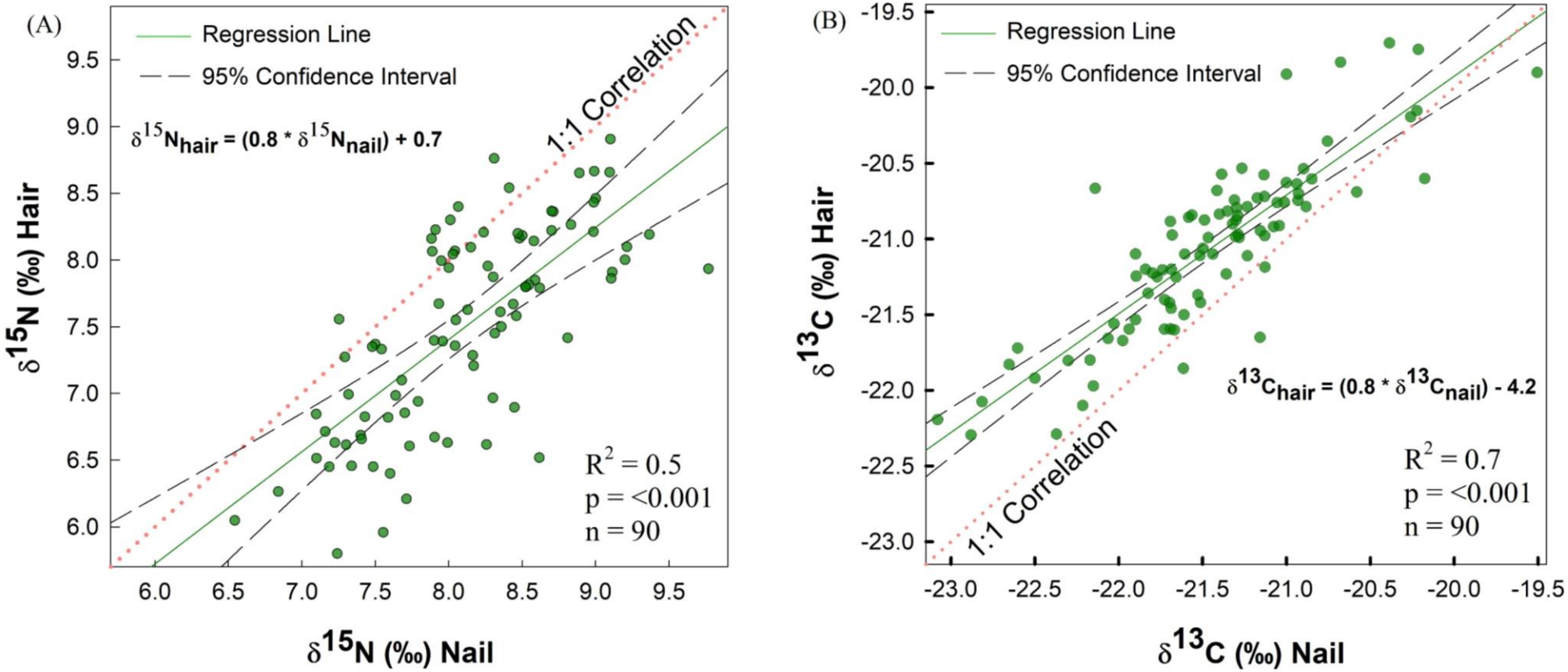
Based on consumption of food item in a particular diet

(A) Individual data points of 66 Food Items of classification 1



Additional Results: 2)

Comparison between scalp hair and fingernails of 90 study participants based on their (A) nitrogen, and (B) carbon stable isotope composition



- $\delta^{15}N$ nail > $\delta^{15}N$ hair

  $\Delta^{15}N_{hair\text{-}nail} = 0.7 \pm 0.7‰$

- $\delta^{13}C$ nail < $\delta^{13}C$ hair

  $\Delta^{13}C_{hair\text{-}nail} = -0.4 \pm 0.3‰$

Due to differing amino acid compositions.

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Thank You