Metabolic iron and copper stable isotope fractionation in mature rhesus macaques (*Macaca mulatta*)

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Introduction

The isotopes of trace metals, such as iron (Fe) and copper (Cu) have been shown to be less in human adult males relative to females, and this sex effect has been linked to female menstruation[1,3,4,5,7]. However, this variation has never been measured in non-human primates. We hypothesized that because rhesus macaques have a similar menstrual cycle to humans, the sex variation in Fe and Cu should be similar to adult humans. To test if there was a similar sex effect in rhesus macaques, we investigated the isotopic fractionation of δ^{56}Fe and δ^{65}Cu of 20 pre- and peri-pubescent rhesus macaques (*Macaca mulatta*) from the Caribbean Primate Research Center, Puerto Rico. Specifically, we examine (1) the potential existence of sex-related differences in Fe and Cu isotope ratios, represented as δ^{56}Fe and δ^{65}Cu, respectively in bulk occipital bone and incisors of identified male and female non-human primates, and (2) variation of Fe and Cu isotope ratios as a factor of age.

Hypotheses

1) The variation of Fe and Cu metabolism between sex that is observed in human bone will be similar in rhesus macaque occipital bone

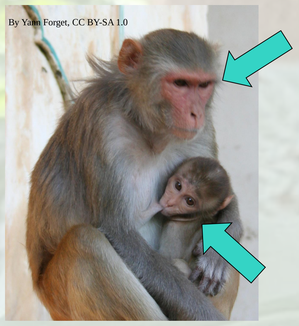
2) The lack of variation of Fe and Cu metabolism between sex observed in human tooth enamel will not be observed in rhesus macaque bulk incisors.

3) Age will not influence δ^{56}Fe and δ^{65}Cu metabolism in rhesus macaque occipital bone and bulk incisors.

Materials & Methods

Materials

- Rhesus Macaques from Caribbean Primate Research Center (CPRC) with known life history traits (DOB, DOD, age, sex)
- 20 individuals (10 males, 10 females)
  - **Females**: 2 juveniles; 8 adults
  - **Males**: 2 juveniles; 8 adults
- 40 samples (100-150mg)
  - 20 incisors (I^1)
  - 20 occipital bone



Methods

- Bone was extracted, crushed, and then reduced to ashes in a muffle furnace at 1,000˚C.
- Then, the ashes were dissolved in 2mL of aqua regia, before undergoing oxidation.
- The samples were processed using anion-exchange chromatography according to the technique of Maréchal and Albarède (2002). The process was repeated twice to remove Ca and P interferences.
- Fe and Cu stable isotope compositions were determined by ThermoScientific Neptune Plus Multiple Collector Inductively-Coupled Plasma Mass Spectrometry (MC-ICP-MS).

Results

Figure 1 No significant results for δ^{56}Fe fractionation in rhesus macaques.

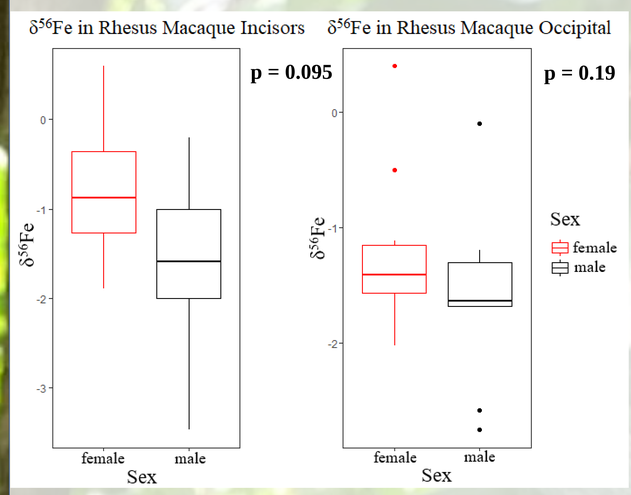


Figure 2 Significant results for δ^{65}Cu fractionation in rhesus macaques.

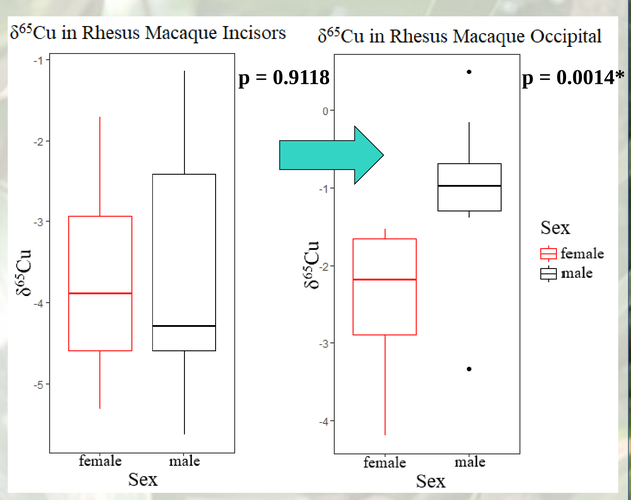


Figure Guide
Significant* $p < 0.05$
Not significant $p > 0.05$

Figure 3 Comparison of the means (Kruskal-Wallis Test) of δ^{65}Cu fractionation between species. Other data was acquired from Jaouen et al. (2012;2013) and Balter et al. (2013).

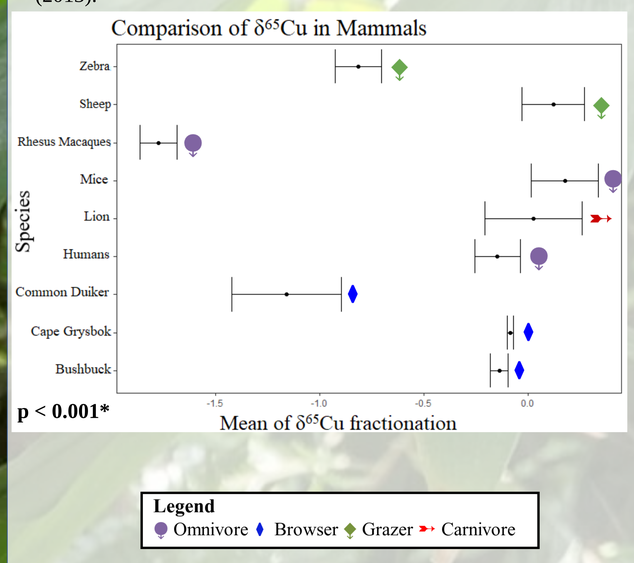
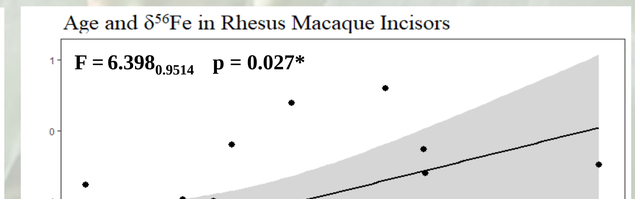


Figure 4 Generalized Additive Model (GAM), where after accounting for age and sex, age predicts δ^{56}Fe fractionation.



Discussion

- Occipital bone and δ^{65}Cu values are <u>useful</u> in indicating differences in trace metal metabolism between sex (*Figure 2*).
- Incisors are <u>not useful</u> in indicating differences in trace metal metabolism between sex, because in rhesus macaques, eruption and formation are pre-pubescent[2].
- In δ^{56}Fe, values <u>increase</u> as males and females age (*Figure 4*).
- Relative to other mammal taxa, on average rhesus macaques, zebras, and common duikers have lower and more individualistic δ^{65}Cu values with a negligible dietary influence (*Figure 3*).
- Since there are no sex differences after accounting for age and sex, menstruation is <u>not</u> the mechanism behind the variation in rhesus macaques.
- Fe and Cu have the potential to be a supplementary tool in archaeology, paleoanthropology, and primatology.

Conclusion

1) We have shown that Cu isotope ratios vary similarly between sex in rhesus macaque occipital bone relative to human bone.

2) We have shown that there is variation between sex in Fe stable isotope ratios as a factor of age in incisors.

3) We have shown that when accounting for age and sex independently, these sex differences are no longer present.

Future Prospects

- We will need to examine the mechanism behind the variation in sex differences in other non-human primate groups.
- We will continue exploring the metabolism of Fe and Cu stable isotopes and their relationship to sex.
- We will need to explore diagenesis and how it affects Fe, Cu, and Zn values in archaeological bone
- We will need to expand the reference dataset for Fe, Cu, and Zn isotope systems in mammals (including humans and hominins).

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