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## Correspondence

# Reply to Van Valkenburgh *et al*.

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Van Valkenburgh and colleagues [1] commented on our previous study in *Current Biology* [2] of Rancho La Brea (California, USA), which contributed stable isotope data from tooth enamel that documented disparate diets in sabertooth cats (*Smilodon fatalis*) and dire wolves (*Canis dirus*) in southern California through the late Pleistocene. We welcome the discourse by Van Valkenburgh *et al*. [1] but contend that many of their criticisms regarding the usefulness of stable isotopes from tooth enamel for inferring animal diets are based on incorrect suppositions in methodology and interpretations. Here, we respond to the critiques of Van Valkenburgh *et al*. [1]. We show that herbivores from Rancho La Brea consumed a mixture of $C_3$ and $C_4$ resources and that enamel carbonate stable isotope data are less prone to diagenesis than bone collagen and are a better representation of whole diet. We also clarify relationships between $\Delta_{carbonate\text{-}collagen}$ offsets and stable isotopes from bone collagen and enamel in dire wolves and extant taxa. We reaffirm our conclusion that *C. dirus* consumed prey from more open environments than *S. fatalis* and the validity of enamel stable isotope data for interpreting the paleoecology of Rancho La Brea.

Van Valkenburgh *et al*. [1] argue that stable isotopes from tooth enamel are less useful than bone collagen because lower first molars mineralize during a shorter window of time than bone and may be influenced by maternal milk. We acknowledge the possibility of a weaning effect in developing teeth but studies that examined the effects of weaning on $\delta^{13}C$ values in tissues, including plasma, hair, and tooth enamel, have shown that the effect is minimal (<1‰ [3]). Furthermore, serial sampling of the enamel of adult upper canines from *S. fatalis* from Rancho La Brea, which developed and mineralized from near birth up to 25 months of age, showed no trend in $\delta^{13}C$ values

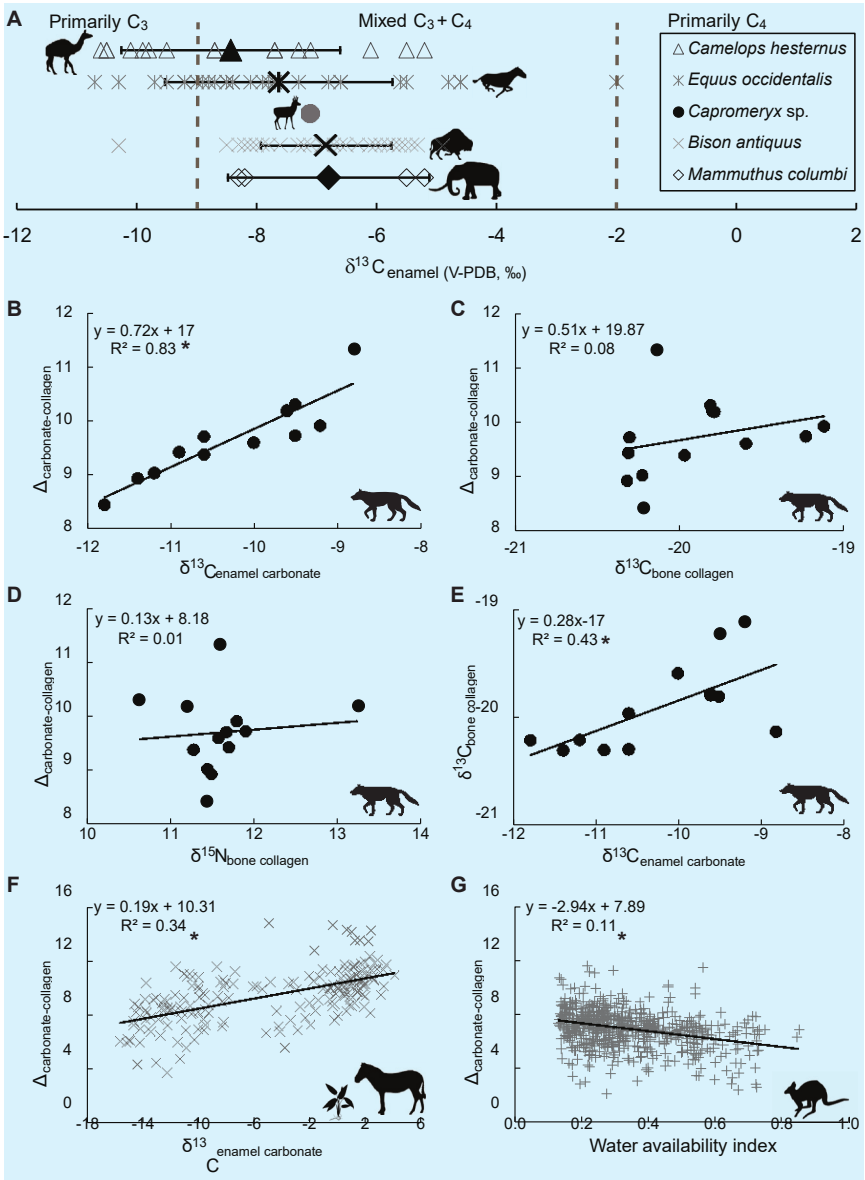


**Figure 1. Summary of carbon isotope data from Rancho La Brea herbivores, *Canis dirus*, and extant herbivores.**

(A) All Rancho La Brea herbivores noted here (taken from [2,5,6]) have mean $\delta^{13}C$ values consistent with a diet of mixed $C_3$ and $C_4$ resources. Dashed lines correspond to approximate demarcations between primarily $C_3$, mixed $C_3$ and $C_4$, and primarily $C_4$ diets. All black symbols denote mean values with error bars noting one standard deviation (n-1). Relationships between $\Delta_{carbonate\text{-}collagen}$ and (B) $\delta^{13}C_{enamel\ carbonate}$ values in *C. dirus*, (C) $\delta^{13}C_{bone\ collagen}$ values in *C. dirus*, and (D) $\delta^{15}N_{bone\ collagen}$ values in *C. dirus* from Rancho La Brea. (E) Relationship between $\delta^{13}C_{enamel\ carbonate}$ values and $\delta^{13}C_{bone\ collagen}$ values in *C. dirus*. Relationship between $\Delta_{carbonate\text{-}collagen}$ and (F) $\delta^{13}C_{enamel\ carbonate}$ values in herbivorous mammals where enamel carbon-ate and bone collagen carbon isotopes have been analyzed, (G) water availability index from kangaroos where enamel carbonate and bone collagen carbon isotopes have been analyzed. Data in B–E ($n$=13) are from [8] and new enamel carbonate data analyzed per [2]. Data in (F) ($n$=190) are from [9] and data in (G) ($n$=680) are from [10]. Asterisks denote significant relationships ($p<0.05$). See also Table S1. (Images: dire wolf from J. Olsson; *Bison*, *Equus*, and *Mammuthus* by Zimices (CC BY-SA 3.0).)

over time and thus no apparent weaning signal [4]. $\delta^{13}C$ values from m1s are separated by 2.9‰ to 3.8‰ between dire wolves and sabertooth cats [2] and only overlap by up to 0.3‰ during any given accumulation [2]. This indicates that the differences observed among these species cannot be explained by weaning alone and implies differences in their dietary ecology. It is possible that these species consumed prey from disparate environments during their youth and competed for the same prey only during adulthood. However, this is improbable based on the ecology of felids and canids today — felids typically use cover to ambush prey while canids rarely utilize vegetation — and on morphological evidence suggesting terrestrial and cursorial hunting, respectively, in these species.

Van Valkenburgh *et al*. [1] questioned the reliability of tooth enamel stable isotope data, asserting that $C_4$ plants were rare and that elevated sloth dentin values are grounds for questioning all enamel data. We note that evidence of $C_4$ vegetation consumption by prey species at La Brea is not debatable [2,5,6] (Figure 1A). Over three decades ago, $C_4$ grass macrofossils (i.e., *Hilaria*, *Bouteloua*) were found in the dental boli of horses from Rancho La Brea. While we agree that we should remain skeptical of tooth dentin-bioapatite from sloths (due to the potential for diagenesis in dentin), enamel stable isotope data should not be disregarded for two reasons: first, it is well established that enamel is less prone to diagenetic alteration than dentin, bone apatite, and bone collagen (bone collagen is organic while enamel is only ~6–10% organic); second, enamel $\delta^{13}C$ values reflect an animal's whole diet (including protein, carbohydrates, fat), while bone collagen $\delta^{13}C$ values primarily reflect dietary protein [7]. If $C_4$ plants are to be incorporated into animal tissues, their presence is more likely to be indicated in enamel, which captures whole diet (including the more abundant carbohydrates present in $C_4$ plants). In carnivores, the consumption of protein and fat will be recorded in tooth enamel — consuming prey with different fat and protein compositions can yield disparate $\delta^{13}C$ carbonate and collagen values in predators.

Van Valkenburgh *et al*. [1] criticized our use of means and standard deviations when using MixSIAR (Bayesian Mixing Models in R). However, this is standard practice and required. Further, we ran two iterations of the model which were both published, one with and one without sloth isotope values (see Figure S1 and Table S3 in [2]). The results from both models do not change our interpretation of data regarding the diets of *S. fatalis* and *C. dirus*.

Since the publication of DeSantis *et al*. [2], we have sampled enamel from the jaws of dire wolves previously sampled for bone collagen [8], using identical methods. There is a significant relationship between enamel carbonate and bone collagen ($p$=0.014, Figure 1E). Notably, these data (Figure 1B–E; Supplemental Information) indicate a positive relationship between $\delta^{13}C$ enamel values and the offset between enamel carbonate and bone collagen (i.e., $\Delta_{carbonate\text{-}collagen}$; $p$<0.001; Figure 1B), similar to herbivores noted in [9] (Figure 2F). Thus, the foraging habitat of prey influences predator $\Delta_{carbonate\text{-}collagen}$ values. It is also possible that water availability (which is often, but not always correlated with enamel $\delta^{13}C$ values) plays a role in influencing $\Delta_{carbonate\text{-}collagen}$ values, as was documented in kangaroos [10] (Figure 2G). The relationship between $\Delta_{carbonate\text{-}collagen}$ offsets is not related to either $\delta^{13}C$ values ($p$=0.333; Figure 1C) or $\delta^{15}N$ values in bone collagen ($p$=0.699; unlike expectations based on a broad literature survey which notes higher $\Delta_{carbonate\text{-}collagen}$ values in herbivores and lower values in carnivores; Figure 2D). We are actively building on these data to clarify the relationship between bone collagen and enamel in carnivores and we stand by our paleoecological interpretations of late Pleistocene Rancho La Brea predators and their prey.

## SUPPLEMENTAL INFORMATION

Supplemental information including one table can be found with this article online at https://doi.org/10.1016/j.cub.2020.01.011.

## AUTHOR CONTRIBUTIONS

L.R.G.D. collected and analyzed new data and wrote the paper, R.S.F., K. F-D., J.M.H., T.E.C, J.M.C., A.B.F., and G.T.T. provided significant input and editorial feedback.

## REFERENCES

1. Van Valkenburgh, B., Clementz, M.T., and Ben-David, M. (2020). Problems with inferring a lack of competition between Rancho La Brea dire wolves and sabertooth cats based on dental enamel. Curr. Biol. *30*, R149–R150.
2. DeSantis, L.R.G, Crites, J.M., Feranec, R.S., Fox-Dobbs, K., Farrell, A.B., Harris, J.M., Takeuchi, G.T., and Cerling, T.E. (2019). Causes and consequences of Pleistocene megafaunal extinctions as revealed from Rancho La Brea mammals. Curr. Biol. *29*, 2488–2495.
3. Tsutaya, T., and Yoneda M. (2015). Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. Am. J. Phys. Anthropol. *156*, 2–21.
4. Wysocki, M.A., Feranec, R.S., Tseng, Z.J., and Bjornsson, C.S. (2015). Using a novel absolute ontogenetic age determination technique to calculate the timing of tooth eruption in the saber-toothed cat, *Smilodon fatalis*. PLoS One *10*, e0129847.
5. Jones, D.B., and DeSantis, L.R.G. (2017). Dietary ecology of ungulates from the La Brea tar pits in southern California: A multi-proxy approach. Palaeogeogr. Palaeoclimatol. Palaeoecol. *466*, 110–127.
6. Feranec, R.S., Hadly, E.A., and Paytan, A. (2009). Stable isotopes reveal seasonal competition for resources between late Pleistocene bison (*Bison*) and horse (*Equus*) from Rancho La Brea southern California. Palaeogeogr. Palaeoclimatol. Palaeoecol. *271*, 153–160.
7. Ambrose, S.H., and Norr, L. (1993). Experimental Evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In Prehistoric Human Bone: Archaeology at the Molecular Level, J.B. Lambert and G. Grupe, eds. (Springer-Verlag Berlin Heidelberg), pp. 1–37.
8. Fox-Dobbs, K., Bump, J.K., Peterson, R.O., Fox, D.L., and Koch, P.L. (2007). Carnivore-specific stable isotope variables and variation in the foraging ecology of modern and ancient wolf populations: case studies from Isle Royale, Minnesota, and La Brea. Can. J. Zool. *85*, 458–471.
9. Codron, D., Clauss, M., Codron, J., and Tütken, T. (2017). Within trophic level shifts in collagen–carbonate stable carbon isotope spacing are propagated by diet and digestive physiology in large mammal herbivores. Ecol. Evol. *8*, 3983–3995.
10. Murphy, B.P., Bowman, D.M.J.S., and Gagan, M.K. (2007). Sources of carbon isotope variation in kangaroo bone collagen and tooth enamel. Geochim. Cosmochim. Acta. *71*, 3847–3858.

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