

# Stable isotopic variations in modern herbivore tooth enamel, plants and water on the Tibetan Plateau: Implications for paleoclimate and paleoelevation reconstructions

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## Abstract

Stable isotope analyses of fossil teeth and other authigenic minerals have been used to reconstruct the paleoenvironment and paleoelevation of the Tibetan Plateau. The accuracy of such reconstructions is limited by the lack of a comprehensive modern comparative database from the region. We analyzed the carbon and oxygen isotopic compositions ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values) of tooth enamel from modern herbivores, the  $\delta^{13}\text{C}$  values of grasses and the  $\delta^{18}\text{O}$  values of water samples collected from various elevations within the Tibetan Plateau to examine their relationships with modern environment/elevation. The  $\delta^{13}\text{C}$  values of enamel samples from horses, yaks and goats display a narrow range of variation, with a mean of  $-10.7 \pm 1.4\%$  ( $n=301$ ), indicating that these modern herbivores were feeding predominantly on C3 plants, consistent with the current dominance of C3 vegetation in the region. Some of the samples have  $\delta^{13}\text{C}$  values between  $-7.3$  and  $-10\%$ . Although these higher  $\delta^{13}\text{C}$  values could suggest consumption of some C4 plants by the animals, the lack of significant seasonal  $\delta^{13}\text{C}$  variations within individual teeth indicates that these higher enamel  $\delta^{13}\text{C}$  values are due to consumption of C3 plants experiencing water stress and/or some CAM plants rather than C4 plants. Our data show that the conservative “cut-off”  $\delta^{13}\text{C}$  value for a pure C3 diet within the Tibetan Plateau should be  $-8\%$  for modern herbivores and  $-7\%$  (or even  $-6.5\%$ ) for fossils if the region was as arid in the past as today. In contrast to the small intra-tooth  $\delta^{13}\text{C}$  variations within individual teeth, serial enamel samples display large intra-tooth  $\delta^{18}\text{O}$  variations, reflecting seasonal variations in the  $\delta^{18}\text{O}$  of meteoric water. The mean  $\delta^{18}\text{O}$  values of tooth enamel from yaks and horses show a strong correlation with water  $\delta^{18}\text{O}$  values, confirming that the  $\delta^{18}\text{O}$  of tooth enamel from obligate drinker generally tracks the  $\delta^{18}\text{O}$  of meteoric water. Unfortunately, elevation alone cannot explain most of the variance in the  $\delta^{18}\text{O}$  of precipitation and tooth enamel, suggesting that quantitative reconstruction of the paleoelevation of the Tibetan Plateau using re-constructed  $\delta^{18}\text{O}$  values of paleo-meteoric water from fossil enamel or other oxygen-bearing minerals is not warranted. For a given environment, horses have the lowest enamel- $\delta^{18}\text{O}$  values while goats display the highest enamel- $\delta^{18}\text{O}$  values among the species studied. The large inter-species  $\delta^{18}\text{O}$  variations are likely due to differences in physiology and diet/drinking behavior of the animals. This underscores the importance of species-specific studies when interpreting  $\delta^{18}\text{O}$  data of fossil mammalian teeth in a stratigraphic sequence as a record of paleoclimate changes.

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## 1. Introduction

The Tibetan Plateau, with an average elevation of about 5 km and an area of  $2.5 \times 10^6$  km<sup>2</sup>, is the most imposing topographic feature on Earth. The uplift of the Tibetan plateau has been

suggested as a driving force on regional and global climates, particularly on Asian monsoon evolution. The timing history of the Tibetan uplift and its effects on Earth's climate and biosphere have been a matter of much debate and speculation (e.g., Kutzbach et al., 1989; Quade et al., 1989; Molnar and England, 1990; Harrison et al., 1992; Prell and Kutzbach, 1992; Zheng et al., 2000; An et al., 2001; Molnar et al., 2006; Rowley and Currie, 2006; Wang et al., 2006). Reconstruction of the paleoclimate and

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the paleoelevation in areas on and around the high plateau is important for understanding the effects of high topography on regional and global climate and ecology, and for testing various models for the timing and mechanism of the Tibetan uplift.

In recent years, several studies have utilized the oxygen isotopic composition of terrestrial carbonates to reconstruct the paleoelevation of the Tibetan Plateau (Garzzone et al., 2000; Rowley et al., 2001; Currie et al., 2005; Rowley and Currie, 2006; DeCelles et al., 2007). One recent study (Rowley and Currie, 2006), based on oxygen isotopic composition of lake and paleosol carbonates, suggests that high elevations of 4000 m or more were attained as early as 40 million years ago, much earlier than previously thought. The underlying principle of this oxygen-isotope-paleoaltimetry approach is that the precipitation (rain or snow) generally becomes increasingly depleted in the heavy oxygen ( $^{18}\text{O}$ ) the higher up a mountain range that it falls — the “altitude effect” (Dansgaard, 1964), and therefore systematic changes in the  $\delta^{18}\text{O}$  of precipitation with elevation could be used to infer the elevation at which the rain or snow fell. This approach also assumes that the  $\delta^{18}\text{O}$  value of paleo-meteoric water can be determined from the  $\delta^{18}\text{O}$  values of oxygen-bearing minerals and that modern precipitation  $\delta^{18}\text{O}$  vs. elevation relationships can be applied to the distant past. However, studies based on carbon isotopic composition of fossil tooth enamel (Wang et al., 2006) and paleobotanical and sedimentary evidence (e.g., Hsü, 1976; Zheng et al., 2000; An et al., 2001) suggest that the present elevation of the plateau was reached much later, in the Plio-Pleistocene, contradicting the interpretations based on carbonate  $\delta^{18}\text{O}$  data (Garzzone et al., 2000; Currie et al., 2005; Rowley and Currie, 2006). There are considerable uncertainties involved in the reconstruction of paleo-meteoric water  $\delta^{18}\text{O}$  values and paleoelevation using the  $\delta^{18}\text{O}$  values of paleosol carbonates and lake carbonates (Hou et al., 2003; Blisnuik and Stern, 2005; Wang et al., 2006). In a region with complex tectonic and climatic history, the interpretation of stable isotopic records is not always straightforward and the knowledge of modern climatic controls, on which these paleoelevation and paleoclimate reconstructions were based, is often inadequate. The problem or inconsistency in the interpretations of different proxies cannot be resolved until we understand how the isotopic compositions of modern precipitation and minerals reflect modern climate, vegetation and elevation.

In this study, we analyzed the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of both bulk and serial tooth enamel samples from modern herbivores (i.e., yaks, goats and horses), the  $\delta^{13}\text{C}$  values of modern grasses and the  $\delta^{18}\text{O}$  and  $\delta\text{D}$  values of water samples collected from various elevations within the Tibetan Plateau. The isotope data, in conjunction with water isotope data published in the literature and other sources, were used to examine how the stable isotope composition of tooth enamel is influenced by altitudinal and seasonal changes in vegetation and precipitation isotopic ratios. The objective of the study is to examine the effects of altitudinal variations in environmental conditions on the stable isotope compositions of modern herbivore tooth enamel and water, and to establish empirical relationships between the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of tooth enamel and the modern environments on the Tibetan Plateau.

## 2. Background

### 2.1. Using carbon isotopes in tooth enamel as paleodietary and paleoecological indicator

Stable carbon isotope analysis of fossil mammalian tooth enamel has been established as a valuable tool for reconstructing paleodiet of herbivores and interpreting paleoenvironmental parameters of terrestrial ecosystems (e.g., Koch, 1998; Kohn and Cerling, 2002). This approach is largely based on the fact that most plants photosynthesize by one of two pathways: the C3 pathway (C3 plants) or the C4 pathway (C4 plants). Plants using these two different pathways have very different stable carbon isotopic ratios. C3 plants, which include trees, most shrubs and cool season grasses, have  $\delta^{13}\text{C}$  values ranging from  $-20\text{‰}$  to  $-35\text{‰}$ , with an average of  $-27\text{‰}$  (O’Leary, 1988; Farquhar et al., 1989; Cerling et al., 1997). Under water-stressed conditions and/or low atmospheric partial pressure of  $\text{CO}_2$  ( $p\text{CO}_2$ ), C3 plants are enriched in  $^{13}\text{C}$  and have  $\delta^{13}\text{C}$  values higher than the average value of  $-27\text{‰}$ . Under closed canopies, C3 plants have lower  $\delta^{13}\text{C}$  values (that can be as low as  $-35\text{‰}$ ) due to the influence of soil respiration (Schleser and Jayasekera, 1985; Sternberg et al., 1989; van der Merwe and Medina, 1989). C4 plants, which are mostly warm season grasses, have  $\delta^{13}\text{C}$  values of  $-9\text{‰}$  to  $-17\text{‰}$ , averaging  $-13\text{‰}$ . The distinct isotopic difference between different photosynthetic-type plants is passed along the food chain to animal tissues with further isotopic fractionation, which can be quantified by standard mass spectrometric techniques. Calcified tissues like bone, tooth enamel and dentine are all inorganic (mineral) and organic composites (Williams and Elliott, 1979). The inorganic phase of calcified tissues is primarily hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), which contains a small amount of structural carbonate substituting for phosphate and hydroxyl ions. Studies of animals of known diet (Lee-Thorp and van der Merwe, 1987; Lee-Thorp et al., 1989; Wang et al., 1994; Cerling et al., 1997) have shown a  $\sim 14\text{‰}$  carbon isotope fractionation between structural carbonate in hydroxyapatite and the diet. Because tooth enamel is almost entirely inorganic and has very low porosity, it is less susceptible to diagenetic alteration than bone or dentine and has been considered the most suitable material for paleoenvironmental study using stable isotopes (Lee-Thorp and van der Merwe, 1987; Ayliffe et al., 1992; Wang and Cerling, 1994). The  $\delta^{13}\text{C}$  values of structural carbonate in tooth enamel can, therefore, be used to determine the proportions of C3 and C4 plants in the mammal’s diet and the types of vegetation available to support them when a variety of animals are analyzed (e.g., Lee-Thorp et al., 1989; Wang et al., 1994; MacFadden and Cerling, 1994; Cerling et al., 1997; Koch, 1998; MacFadden et al., 1999; Kohn and Cerling, 2002; Wang and Deng, 2005; Wang et al., 2006). Animals that eat C3 plants typically have enamel- $\delta^{13}\text{C}$  values around  $-13\text{‰}$ ; C4 grazers have  $\delta^{13}\text{C}$  values around  $+1\text{‰}$ ; and mixed feeders that eat both C3 and C4 plants fall somewhere in between these two extremes (Lee-Thorp et al., 1989; Cerling et al., 1997). Some variability (up to several per mil) can be expected due to responses of plants to changes in environmental parameters such as soil moisture and

atmospheric  $p\text{CO}_2$  (e.g., Farquhar et al., 1989; Tieszen, 1991; Cerling et al., 1997, 2004).

## 2.2. Using oxygen isotopic composition of tooth enamel as paleoclimate indicator

Oxygen isotopes in tooth enamel contain valuable information about climate (e.g., Longinelli, 1984; Ayliffe and Chivas, 1990; Ayliffe et al., 1992; Bryant et al., 1994; Bryant et al., 1996; Fricke and O'Neil, 1996; Fricke et al., 1998a,b; Koch, 1998; Sponheimer and Lee-Thorp, 1999; Wang and Deng, 2005; Hoppe, 2006). Since mammalian bone and tooth hydroxyapatite are formed at constant body temperature which is not affected by environmental temperature variations,  $\delta^{18}\text{O}$  of biogenic apatite in bone and tooth is directly related to the  $\delta^{18}\text{O}$  of body water (Longinelli, 1984; Luz et al., 1984; Nagy, 1989; D'Angela and Longinelli, 1990; Iacumin et al., 1996). The  $\delta^{18}\text{O}$  of body water is controlled by a number of variables including the  $\delta^{18}\text{O}$  of drinking water and water in food, physiological processes, dietary/drinking behavior and so forth (Longinelli, 1984; Luz et al., 1984; Bryant and Froelich, 1995; Kohn, 1996). Studies have shown that  $\delta^{18}\text{O}$  values of tooth enamel from large animals (> 1 kg) carry information about meteoric water because most of an animal's ingested water comes from meteoric water (Longinelli, 1984; Luz et al., 1984; Bryant et al., 1996). Because the  $\delta^{18}\text{O}$  of meteoric water is sensitive to climatic variables such as temperature, seasonality of rain, and the amount of rain (Dansgaard, 1964; Rozanski et al., 1993), the  $\delta^{18}\text{O}$  of tooth enamel is a complex function of climate and has been used as a proxy for paleoclimatic conditions during tooth growth (e.g., Longinelli, 1984; Koch et al., 1989; Bryant et al., 1994; Bryant et al., 1996; Wang and Deng, 2005).

Furthermore, mammalian tooth enamel forms incrementally from the crown to the base of the tooth, recording a series of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values over time. These values can vary along the growth axis within a single tooth up to several per mils. Since mammalian teeth normally form over a period of 1 to 2 years, serial enamel samples from individual teeth provide an excellent record of seasonal variations in diet and climate during tooth growth (e.g., Koch et al., 1995; Fricke and O'Neil, 1996; Fricke et al., 1998a; Sharp and Cerling, 1998; Dettman et al., 2001; Balassee et al., 2003; Nelson, 2005; Sponheimer et al., 2006).

## 3. Materials and methods

Modern herbivore teeth, grasses and water samples were collected from various elevations within the Tibetan Plateau in the summers of 2004, 2005, and 2006 for stable carbon (C), oxygen (O) and hydrogen isotopic analyses. The teeth come from domesticated herbivores including yaks (*Bos grunniens*), goats (*Capra hircus*), and horses (*Equus caballus*), with the exception of one sample from a wild Tibetan ass (*Equus kiang*). The tooth samples were cleaned by scraping any dirt or other material off the enamel. Bulk enamel samples were taken by drilling down the tooth along the growth axis. Serial samples were collected by drilling in bands perpendicular to the growth axis. The enamel samples were then prepared following a

treatment procedure described in Wang and Deng (2005). The treated enamel samples were reacted with 100% phosphoric acid at 25 °C for about 72 h and the carbon and oxygen isotopic ratios of the  $\text{CO}_2$  produced were analyzed using a Gas Bench II Auto-carbonate/water device connected to a Finnigan MAT Delta Plus XP stable isotope ratio mass spectrometer (IRMS) at the Florida State University (FSU). Water samples were analyzed using the equilibration methods (Thermo Finnigan Operating Manual). 500 micro-liter ( $\mu\text{L}$ ) of sample was injected into an open vial and then a platinum catalyst rod was inserted in the vial. The vial was then sealed with a new septum. Vials containing water samples and standards were then loaded in the sample tray of the Gas Bench II Auto-carbonate/water device. Residual air in the vials was removed by flushing the vials with He containing 2%  $\text{H}_2$  which was used as an equilibration gas. Hydrogen isotope measurements were carried out after an equilibration time of 40 min (at 25 °C) using the Gas Bench II Auto-carbonate/water device connected to the IRMS. After the hydrogen isotope analysis was completed, the vials containing the samples and standards were flushed with He containing 0.3%  $\text{CO}_2$ . The 0.3%  $\text{CO}_2$  was used as an equilibration gas. After an equilibration time of 24 h at 25 °C, the oxygen isotope ratios were measured using the Gas Bench II Auto-carbonate/water device connected to the IRMS. Plant samples were dried and ground into powder. Their stable carbon isotopic compositions were measured using a Carlo Erba Elemental Analyzer interfaced to the IRMS at FSU. Results are reported in standard delta ( $\delta$ ) notation (i.e.,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and  $\delta\text{D}$ ) in reference to the international carbonate standard VPDB and water standard VSMOW. The analytical precision (based on replicate analyses of NBS-19 and VSMOW and several other lab standards processed with each batch of samples) is  $\pm 0.1\%$  or better for both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ , and  $\pm 1\%$  for  $\delta\text{D}$ .

## 4. Results

### 4.1. $\delta^{13}\text{C}$ of modern plants

Sixty-one plant samples (primarily grasses) were collected spanning an elevation range of ~2700 m to ~5400 m above sea level (a.s.l.) within the Tibetan Plateau in the summers of 2004 and 2005. Carbon isotope analyses of these samples show that only four samples are C4 grasses (Fig. 1a). Two of the C4 grasses were found in the town Little Gyirong near the Nepal-China border at about 2700 m a.s.l., and the other two samples were found along side of the main highway in southern Tibet, near villages at 4000 m and 4100 m a.s.l., respectively. Although many of the grass samples were not identified into species, three of the four C4 grasses clearly belong to the same species except that the samples found at higher elevations were a lot smaller compared to the one collected at 2700 m elevation. We also found a C4 shrub in the Qaidam Basin on the northern Tibetan Plateau at about 2900 m a.s.l. which was identified as *Halocnemum strobilaceum* (Pall.) M.B and has a  $\delta^{13}\text{C}$  of  $-13.3\%$  (Fig. 1b). The  $\delta^{13}\text{C}$  values of C3 plants collected from the Tibetan Plateau range from  $-22.2\%$  to  $-28.3\%$ , with a mean of  $-25.7 \pm 1.4\%$  ( $n=56$ ); the C4 grasses have a mean  $\delta^{13}\text{C}$  value of  $-13.1 \pm 0.8\%$  ( $n=4$ ). As shown in Fig. 1b, the

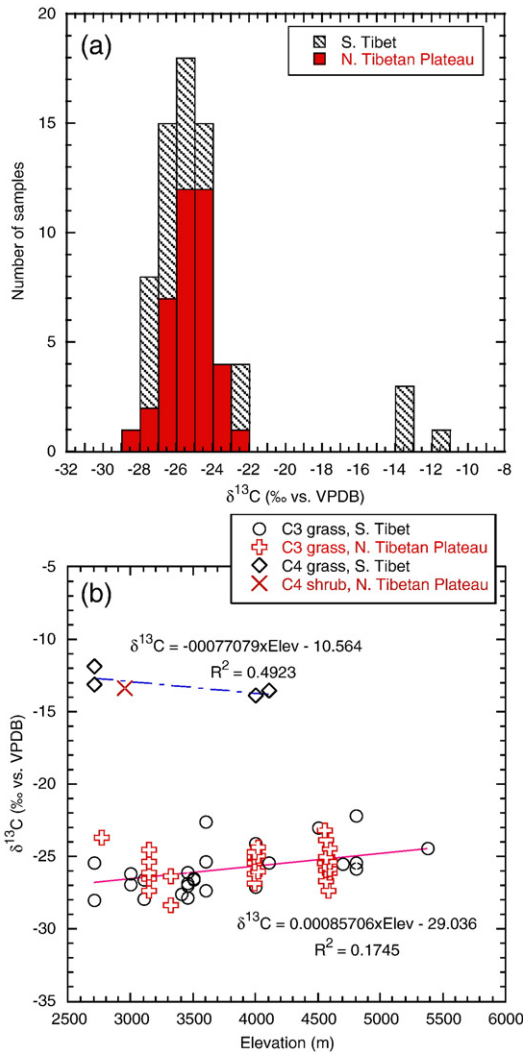


Fig. 1.  $\delta^{13}\text{C}$  values of grass samples collected from the Tibetan Plateau (a) and their relationships with elevation (b). A C4 shrub found in the Qaidam Basin on the northern Tibetan Plateau is also plotted in (b) for comparison. The positive correlation between the  $\delta^{13}\text{C}$  of C3 plants and elevation is similar to (but weaker than) what was observed in C3 plants along an altitudinal transect in the Andes (Tieszen, 1991), most likely reflecting the combined effect of high water stress on the Tibetan Plateau and decreasing atmospheric  $p\text{CO}_2$  with increasing altitude. The opposite plant- $\delta^{13}\text{C}$  vs. elevation trends observed in C4 and C3 plants are consistent with model predictions (Farquhar et al., 1989) that changes in atmospheric  $p\text{CO}_2$  have exactly opposite effects on C isotopic fractionation in C4 and C3 plants.

$\delta^{13}\text{C}$  values of C3 plants appear to display a positive correlation with elevation while the  $\delta^{13}\text{C}$  values of C4 plants show a negative correlation with elevation. These trends most likely reflect the combined effect of high water stress on the Tibetan Plateau and decreasing atmospheric  $p\text{CO}_2$  with increasing altitude (Farquhar et al., 1989; Tieszen, 1991).

#### 4.2. Isotopic composition of precipitation and surface waters

Meteorological and isotopic observations on the Plateau are relatively few and of short duration. There is only one IAEA-GNIP (International Atomic Energy Agency Global Network for Isotopes in Precipitation) station on the Plateau, which is

located in Lhasa ( $29^\circ 42' \text{N}$ ,  $91^\circ 08' \text{E}$ ; 3649 m). As shown in Fig. 2, the water samples that we collected in the summers of 2004, 2005, and 2006 plot on or near the Global Meteoric Water Line (GMWL) with the exception of lakes and ponds which are all plotted below the GMWL, indicating that lakes and ponds in the region have been severely affected by evaporation (Gonfiantini, 1986). In addition to our own data, we compiled published precipitation isotope data for the Tibetan region from the literature (Thompson et al., 1989; Bartarya et al., 1995; Thompson et al., 1997; Wei and Gasse, 1999; Thompson et al., 2000; Kang et al., 2002; Hou et al., 2003; Thompson et al., 2003; Gajurel et al., 2006) and extracted weighted annual means of  $\delta^{18}\text{O}$  in precipitation at Lhasa and several other IAEA-GNIP stations in the Asian summer monsoon region from the IAEA-GNIP database ([http://www-naweb.iaea.org/napc/ih/GNIP/IHS\\_GNIP.html](http://www-naweb.iaea.org/napc/ih/GNIP/IHS_GNIP.html)). All water isotope data are shown in Fig. 3. It has been recognized that there is a large spatial and temporal variability in the  $\delta^{18}\text{O}$  of precipitation in the Asian monsoon region (Araguas-Araguas et al., 1998; Johnson and Ingram, 2004; Vuille et al., 2005). Although precipitation generally has higher  $\delta^{18}\text{O}$  values at low elevation sites than at high elevation sites (Fig. 3b), the  $\delta^{18}\text{O}$  values of precipitation in the Tibetan region do not show a strong correlation with elevation from near sea level to 3000 m a.s.l. ( $R^2 = 0.0683$  for linear fit to the data) and also for elevations above 3000 m ( $R^2 = 0.3150$  for linear fit to the data). That is, elevation alone cannot explain most of the variance in the  $\delta^{18}\text{O}$  of precipitation in this region (Fig. 3b).

#### 4.3. Carbon and oxygen isotopic compositions of modern herbivore tooth enamel

One hundred and twelve individual teeth from modern goats, horses, and yaks from the Tibetan Plateau were collected for this study. About 300 bulk and serial enamel samples were obtained

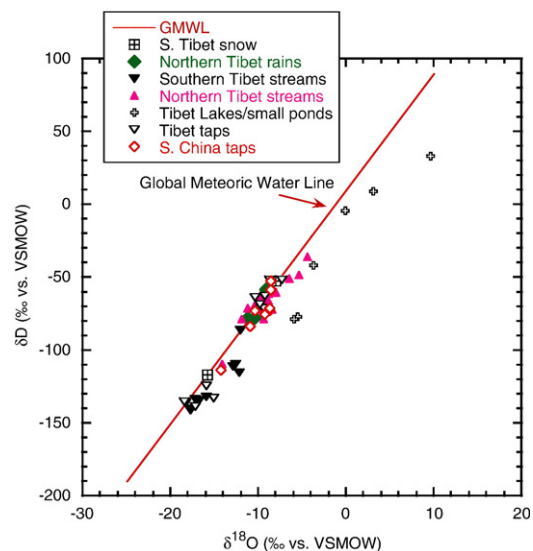


Fig. 2.  $\delta\text{D}$  and  $\delta^{18}\text{O}$  values of water samples collected in the summers of 2004, 2005 and 2006. It shows that most of the water samples are plotted on or close to the Global Meteoric Water Line (GMWL) except lakes and ponds which are all plotted below the GMWL, indicating that lakes and ponds in the region have been severely affected by evaporation.

from these modern teeth for carbon and oxygen isotope analysis. The results are summarized in Table 1 and Fig. 4.

The carbon isotopic composition of tooth enamel from modern herbivores within the Tibetan Plateau displays a narrow range of variation (Table 1). The  $\delta^{13}\text{C}$  values of enamel samples from horses range from  $-10.6\text{‰}$  to  $-13.9\text{‰}$ , with an average  $\delta^{13}\text{C}$  value of  $-11.9 \pm 0.7\text{‰}$ . The  $\delta^{13}\text{C}$  values of yak tooth enamel range from  $-7.3\text{‰}$  to  $-14.2\text{‰}$ , averaging  $-10.1 \pm 1.3\text{‰}$ .

The goat teeth have  $\delta^{13}\text{C}$  values ranging from  $-7.5\text{‰}$  to  $-12.1\text{‰}$ , with a mean of  $-10.1 \pm 1.2\text{‰}$ . The  $\delta^{18}\text{O}$  values of bulk tooth enamel samples, on the other hand, vary widely within and between species (Table 1). The serial enamel samples from selected teeth from both southern and northern Tibetan Plateau show small intra-tooth  $\delta^{13}\text{C}$  variations ( $< \sim 4\text{‰}$ ) within individual teeth. In contrast,  $\delta^{18}\text{O}$  values display large intra-tooth variations (up to  $9.3\text{‰}$ ) within individual teeth (Fig. 5).

## 5. Discussions

### 5.1. Vegetation $\delta^{13}\text{C}$ variability and diets of modern herbivores

In the Tibetan region, the vegetation and climate change with elevation. Precipitation generally decreases from south/southeast to north/northwest and mean annual temperatures decrease with increasing elevation. Changes in precipitation and temperature

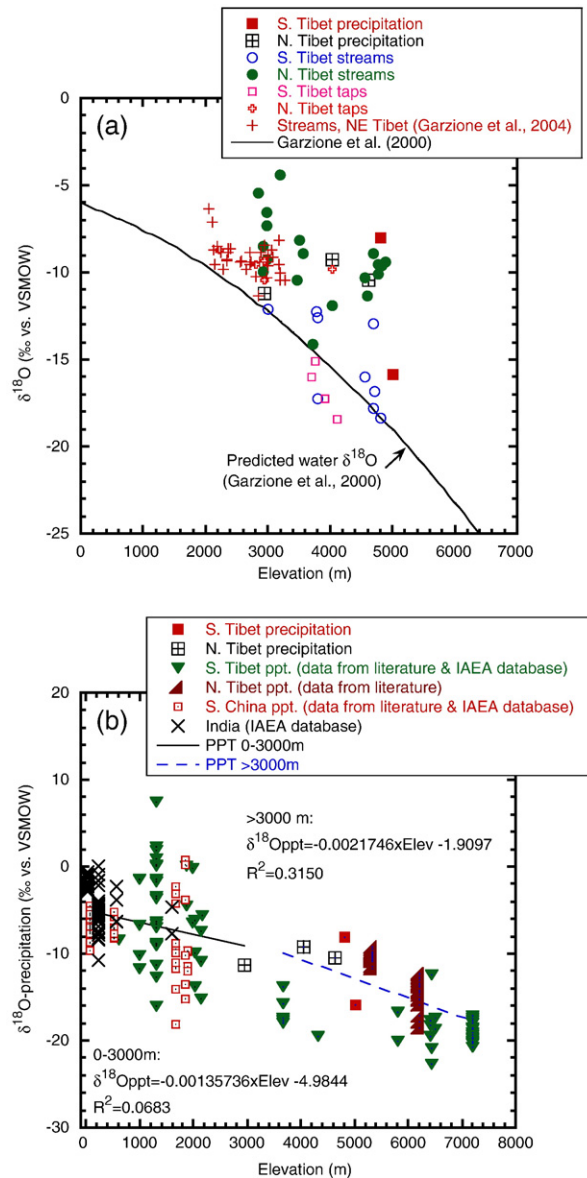


Fig. 3. Variations in the  $\delta^{18}\text{O}$  values of (a) water samples collected in this study with elevation (also plotted in the diagram are the water- $\delta^{18}\text{O}$  vs. elevation relation given in Garzzone et al. (2000) and stream water  $\delta^{18}\text{O}$  data from Garzzone et al. (2004) for comparison), and (b) precipitation with elevation in the Tibetan region as well as in south China and India within the Asian summer monsoon regime.

Table 1  
The mean and range of variations of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of tooth enamel from modern herbivores on the Tibetan Plateau

Species	Location	Elevation (m a.s.l.)	Mean $\delta^{13}\text{C}$ -enamel (% vs. VPDB)	Range of variation of bulk $\delta^{13}\text{C}$ values	Mean $\delta^{18}\text{O}$ -enamel (% vs. VPDB)	Range of variation of bulk $\delta^{18}\text{O}$ values	Number of enamel samples	Number of teeth
<i>S. Tibetan Plateau</i>								
Goat								
<i>Capra hircus</i>	Ladong Village	3700	-10.7±0.6	-9.8 to -11.4	-6.2±3.1	-1.8 to -10.0	8	8
<i>Capra hircus</i>	Near Lazi	3900	-11.3		-10.7		1	1
<i>Capra hircus</i>	Gyirong	4100	-9.3±1.3	-7.7 to -12.1	-8.8±5.0	-4.6 to -15.4	23	12
<i>Capra hircus</i>	Jiajie Port	4700	-9.7±0.8	-8.7 to -10.6	-12.0±2.0	-9.1 to -14.5	5	5
Horse								
<i>Equus caballus</i>	Gyirong	4100	-12.0±1.1	-10.8 to -13.9	-16.8±2.1	-14.8 to -20.0	24	14
Yak								
<i>Bos grunniens</i>	Little Gyirong	2700	-12.6±0.6	-11.8 to -13.2	-8.8±1.5	-7.4 to -11.2	6	6
<i>Bos grunniens</i>	Bailang Co.	3800	-11.0		-16.1		1	1
<i>Bos grunniens</i>	Jiangzi Co.	3900	-10.1±1.5	-7.3 to -12.1	-13.2±3.6	-8.5 to -17.0	38	15
<i>Bos grunniens</i>	Gyirong	4100	-10.2±1.2	-9.4 to -14.2	-14.6±1.8	-12.4 to -19.2	52	17
<i>Bos grunniens</i>	Gyirong	4200	-12.5		-8.6		1	1
<i>Bos grunniens</i>	Jiajie Port	4700	-9.5±0.8	-8.1 to -11.7	-14.0±1.3	-12.3 to -16.0	46	24
<i>N. Tibetan Plateau</i>								
Goat								
<i>Capra hircus</i>	Xi-Da-Tan	4123	-10.9±0.6	-10.6 to -11.2	-6.5±2.7	-4.2 to -7.9	25	2
Horse								
<i>Equus caballus</i>	Kunlun Pass	4700	-11.9±0.7	-11.5 to -12.2	-8.0±1.3	-7.2 to -9.2	68	4
<i>Equus kiang</i>	Kunlun Pass	4700	-11.5		-9.2		1	1
Yak								
<i>Bos grunniens</i>	Kunlun Pass	4800	-12.4		-6.8		1	1

with elevation result in a distinct zonal vegetation pattern (Lu et al., 2004). In southern Tibet where most of the herbivore teeth were collected, forests grow in valleys and mountain slopes below ~3500 m. The zone between 3500 and 4000 m is occupied by subalpine shrub-meadow, and alpine meadow and alpine desert occur above 4000 m (Lu et al., 2004). In northern Tibetan Plateau, ecosystems consist primarily of deserts and alpine meadows. In the Tibetan region, the forest and meadow communities are dominated by C3 plants whereas the desert is dominated by CAM plants (Lu et al., 2004). Carbon isotope analysis of soil organic matter (SOM) in Tibetan soils (Lu et al., 2004) has shown that the  $\delta^{13}\text{C}$  of SOM, which represents the average  $\delta^{13}\text{C}$  value of the plant community, generally increases from forest ( $-25.9\pm 1.2\text{‰}$ ) to shrub ( $-24.7\pm 1.4\text{‰}$ ), steppe ( $-23.1\pm 1.3\text{‰}$ ), alpine meadow

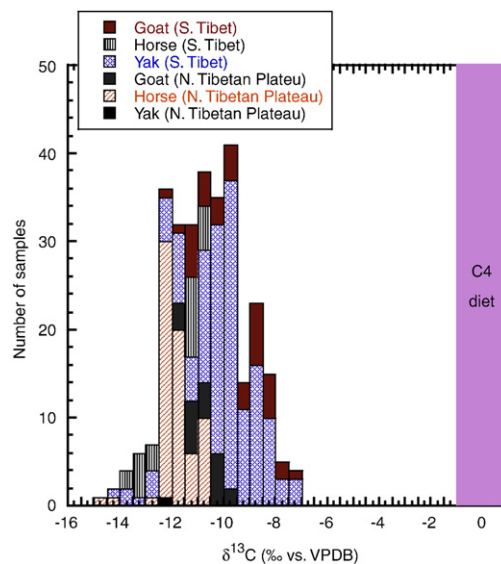


Fig. 4.  $\delta^{13}\text{C}$  values of structural carbonate in tooth enamel (including both bulk and serial samples) from modern herbivores within the Tibetan plateau.

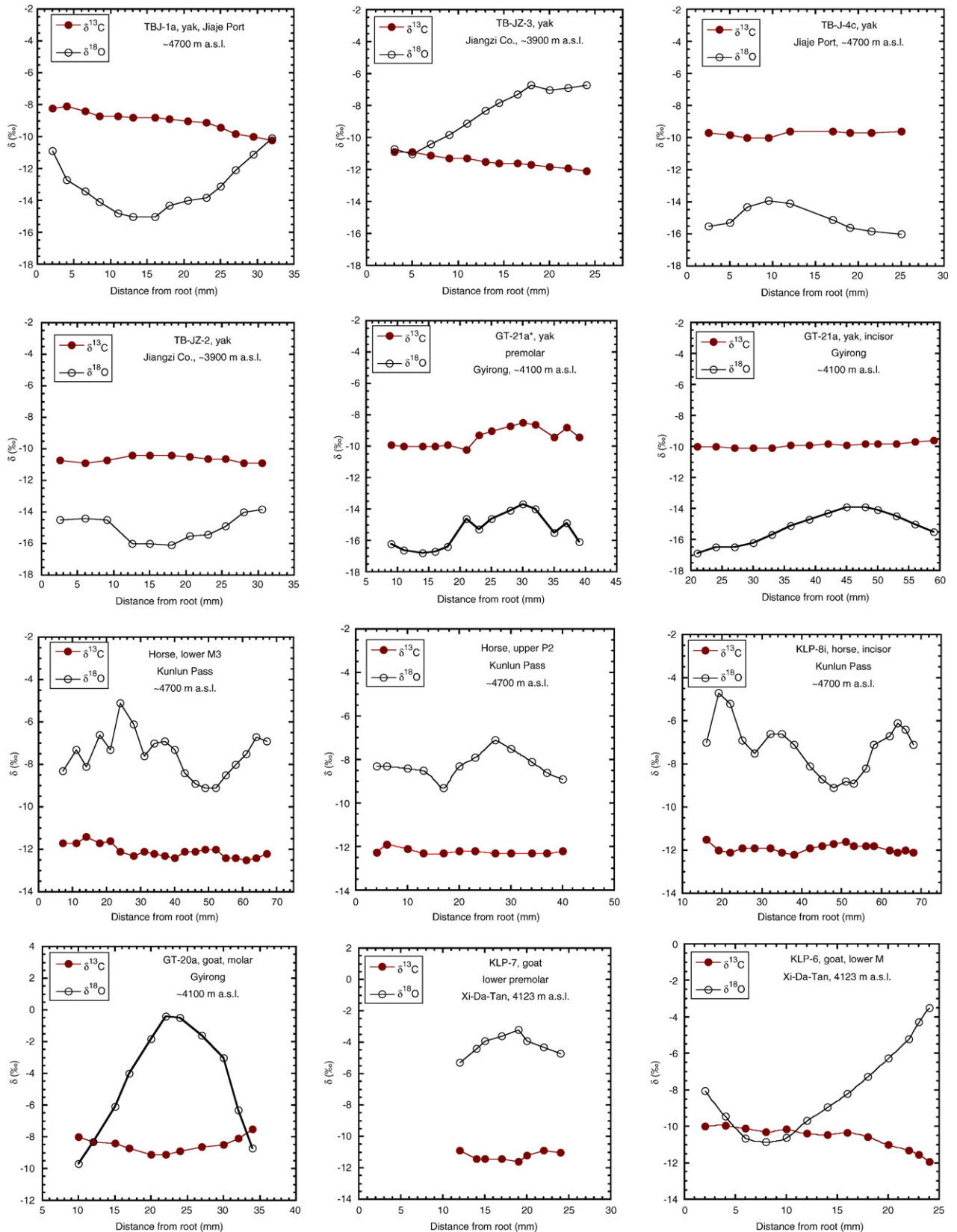


Fig. 5. Intra-tooth  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  variations within individual herbivore teeth from the Tibetan Plateau.

( $-23.6 \pm 0.7\text{‰}$ ), alpine desert steppe ( $-21.3 \pm 1.6\text{‰}$ ), and alpine desert ( $-18.9 \pm 2.5\text{‰}$ ), reflecting changes in climatic conditions (i.e., mean annual precipitation and temperature) and species composition. Recently, C4 grasses were discovered in the warmest months on the Tibetan Plateau, but they account for negligible amounts of the biomass (e.g., Wang et al., 2004; Deng and Li,

2005). Our survey of grasses in the Tibetan region (Fig. 1) confirms this earlier finding. Because the few C4 grasses were found near villages and the main highway, we suspect that humans may have played a role in spreading C4 grass seeds to higher elevations. C3 grasses that we collected from the region have a mean  $\delta^{13}\text{C}$  value of  $-25.7 \pm 1.4\text{‰}$  (Fig. 1), higher than the average  $\delta^{13}\text{C}$  value for C3 plants due to the influence of high water stress and low  $p\text{CO}_2$  at high altitudes (Farquhar et al., 1989; Tieszen, 1991).

The  $\delta^{13}\text{C}$  values of tooth enamel from modern herbivores on the Tibetan Plateau (Fig. 4) range from  $-7.3$  to  $-14.2\text{‰}$  with a mean of  $-10.7 \pm 1.4\text{‰}$  ( $n=301$ ). Some of the samples have  $\delta^{13}\text{C}$  values higher than  $-10\text{‰}$ . Although these higher  $\delta^{13}\text{C}$  values could suggest consumption of some C4 plants by the animals, the lack of significant seasonal variations within individual teeth (Fig. 5) indicates that these higher enamel  $\delta^{13}\text{C}$  values are most likely due to consumption of C3 plants experiencing water and  $\text{CO}_2$  stress and/or some CAM plants rather than intake of C4 grasses whose availability would be highly seasonal. In the Asian monsoon region, which includes our study area, summer precipitation has lower  $\delta^{18}\text{O}$  values than winter precipitation (Araguas-Araguas et al., 1998; Johnson and Ingram, 2004). That is, the lower enamel- $\delta^{18}\text{O}$  values within a tooth would correspond to summer months and higher  $\delta^{18}\text{O}$  values would represent winter months. If the high enamel- $\delta^{13}\text{C}$  values were due to consumption of C4 plants, one would expect to see significant intra-tooth  $\delta^{13}\text{C}$  variations with higher  $\delta^{13}\text{C}$  values corresponding to lower  $\delta^{18}\text{O}$  values (representing enamel formed in summer months) within individual teeth. The samples analyzed here do not show this pattern (Fig. 5). The lack of significant seasonal  $\delta^{13}\text{C}$  variations within individual teeth indicate that C4 grasses were not an important component of these modern herbivores' diets. Because tooth enamel is enriched in  $^{13}\text{C}$  by  $\sim 14\text{‰}$  relative to the diet (Cerling et al., 1997), the  $\delta^{13}\text{C}$  values of tooth enamel from the Tibetan Plateau correspond to a dietary intake of  $-21.3$  to  $-28.2\text{‰}$ , with a mean value of  $-24.7 \pm 1.4\text{‰}$ . These estimated diet  $\delta^{13}\text{C}$  values are within the  $\delta^{13}\text{C}$  range for modern C3 plants. Thus, enamel- $\delta^{13}\text{C}$  values of modern herbivores accurately reflect the modern C3-dominated environment of the Tibetan Plateau. Our data also show that the "cut-off" enamel  $\delta^{13}\text{C}$  value for a pure C3 diet for modern herbivores on the Tibetan Plateau should be  $-8\text{‰}$  or perhaps even as high as  $-7.3\text{‰}$  because of highly water-stressed conditions. Unfortunately, the few samples that yielded  $\delta^{13}\text{C}$  values between  $-8\text{‰}$  and  $-7.3\text{‰}$  were too damaged to permit serial sampling. Isotopic analysis of serial samples could help determine if C4 grasses were a part of the diet.

## 5.2. Variations of enamel $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values within and between species

The bulk enamel samples show variations in carbon and oxygen isotope composition within a species and also between the different species (Table 1). The largest intra-population  $\delta^{13}\text{C}$  variations are  $\sim 3\text{‰}$  for horses,  $\sim 4\text{‰}$  for goats, and  $\sim 5\text{‰}$  for yaks (Table 1). These  $\delta^{13}\text{C}$  variations reflect variations in the C isotopic compositions of plants consumed by the animals and dietary preferences of the animals. Inter-tooth variations in  $\delta^{13}\text{C}$  within an individual are small (Fig. 6). The largest variation seen is  $\sim 4\text{‰}$  between a P3 and P4 from a yak (Fig. 6). The inter-tooth  $\delta^{13}\text{C}$  variation reflects variations in the  $\delta^{13}\text{C}$  values of plants consumed by the individual during the time these teeth were mineralizing.

In contrast to  $\delta^{13}\text{C}$  values, inter-tooth variations in  $\delta^{18}\text{O}$  within an individual are generally larger than the variations in  $\delta^{13}\text{C}$ , with some variations up to  $8.2\text{‰}$  (Fig. 6). Because different teeth are formed at different times of an animal's life (Hoppe et al., 2004), the large inter-tooth  $\delta^{18}\text{O}$  variation within an individual most likely reflects large seasonal and/or annual variations in the  $\delta^{18}\text{O}$  of water ingested by the animal. The "nursing effect" could also contribute to the large inter-tooth  $\delta^{18}\text{O}$  variation. Foals rarely drink water before they are weaned. Thus, teeth formed before or during weaning (e.g., M1 and M2) may show a larger  $^{18}\text{O}$ -enrichment relative to local meteoric water than teeth formed after weaning such as M3, P2, P3 and P4 (Bryant et al., 1996). However, our data do not show a consistent enrichment pattern in milk teeth (i.e., M1 and M2) compared to other permanent teeth (Fig. 6). This may be caused by the large seasonal variations in the  $\delta^{18}\text{O}$  values of local water that overwhelmed the relatively small "nursing effect".

There are large  $\delta^{18}\text{O}$  variations within and between species (Table 1). The intra-population  $\delta^{18}\text{O}$  variations are  $5.2\text{‰}$  or less for horses,  $8.5\text{‰}$  or less for yaks, and  $10.8\text{‰}$  or less for goats (Table 1). The largest  $\delta^{18}\text{O}$  variation is seen in goats in the Gyirong Basin, with a range of variation of  $10.8\text{‰}$ . These variations are larger than within population variability reported previously for several north American mammals (Clementz and Koch, 2001; Hoppe et al., 2004; Hoppe, 2006). In a given environment, the horse samples have the lowest  $\delta^{18}\text{O}$  values, while the goat samples are the most  $^{18}\text{O}$ -enriched (Table 1, Fig. 7b). The inter-species  $\delta^{18}\text{O}$  variations are caused by differences in physiology, diet and drinking behavior of different animals (Bryant and Froelich, 1995; Kohn et al., 1996; Kohn, 1996). Both theoretical and empirical studies suggest that the  $\delta^{18}\text{O}$  of enamel is directly related to the  $\delta^{18}\text{O}$  of body water, which for large mammals is mostly derived from two sources: meteoric water and food (Luz et al., 1984; Longinelli, 1984; Koch et al., 1989; Bryant et al., 1996; Kohn, 1996; Fricke et al., 1998a; Sponheimer and Lee-Thorp, 1999). Leaf water is generally enriched in  $^{18}\text{O}$  relative to local meteoric water due to preferential loss of the isotopically lighter water molecule  $\text{H}_2^{16}\text{O}$  during evapotranspiration, and this  $^{18}\text{O}$ -enrichment is substantial in arid regions but decreases with increasing relative humidity (Dongmann et al., 1974; Epstein et al., 1977; Yakir, 1992). Thus, obligate drinkers such as horses would have lower  $\delta^{18}\text{O}$  values than animals like goats that obtain a larger proportion of their body water from plant sources (e.g., leaves, fruits) in a given ecosystem. The larger intra-species  $\delta^{18}\text{O}$  variations seen in goats likely reflect large variations in leaf water  $\delta^{18}\text{O}$  in arid environments that dominate the Tibetan Plateau.



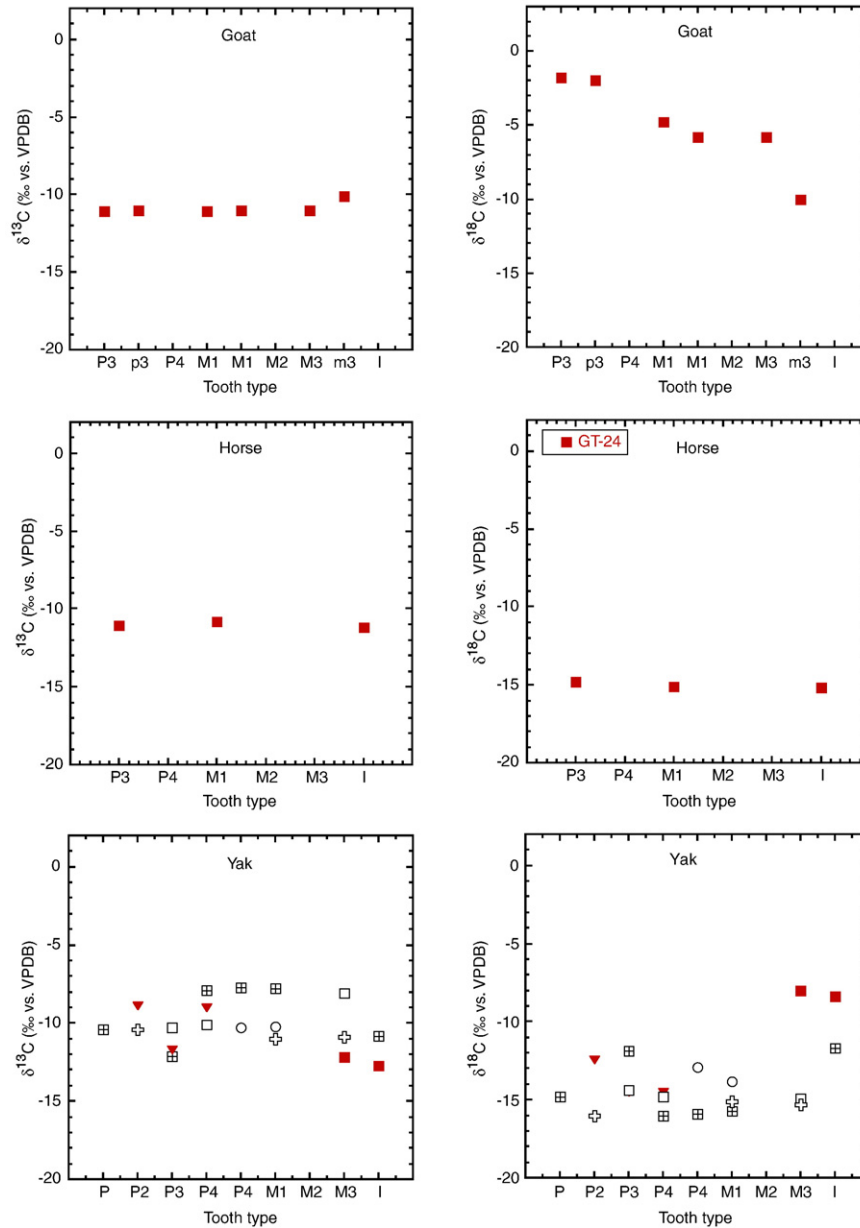


Fig. 6. Inter-tooth carbon and oxygen isotopic variations in goat, horse and yak. Different symbols represent different individuals.

Serial samples show small  $\delta^{13}\text{C}$  variations ( $\sim 4\%$  or less) within each tooth, but large intra-tooth  $\delta^{18}\text{O}$  variations (Fig. 5). The observed small intra-tooth  $\delta^{13}\text{C}$  variations primarily reflect variations in the  $\delta^{13}\text{C}$  of C3 plants consumed by the animals rather than the intake of C4 grasses as discussed in the previous section. The large intra-tooth  $\delta^{18}\text{O}$  variations reflect seasonal variations in the oxygen isotopic composition of precipitation that provides drinking water for the animals and water for plants consumed by the animals.

### 5.3. Climatic controls on enamel $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variability

The  $\delta^{13}\text{C}$  values of tooth enamel from southern Tibet appear to show a positive trend with increasing elevation ( $R^2=0.3$  for linear fit to the mean  $\delta^{13}\text{C}$  values), generally consistent with altitudinal variations in the  $\delta^{13}\text{C}$  of the plant community (Lu et al., 2004), whereas no trends are apparent when samples from the northern Tibetan Plateau are included (Fig. 7a). In contrast, the  $\delta^{18}\text{O}$  values of tooth enamel from either southern or northern Tibetan Plateau do not show a clear trend with increasing elevation (Fig. 7b). The  $\delta^{18}\text{O}$  values of tooth enamel from yaks and horses show a strong positive correlation with the  $\delta^{18}\text{O}$  values of water (Fig. 8a). The correlation becomes stronger when the mean enamel- $\delta^{18}\text{O}$  values from yaks and horses are used instead of individual enamel- $\delta^{18}\text{O}$

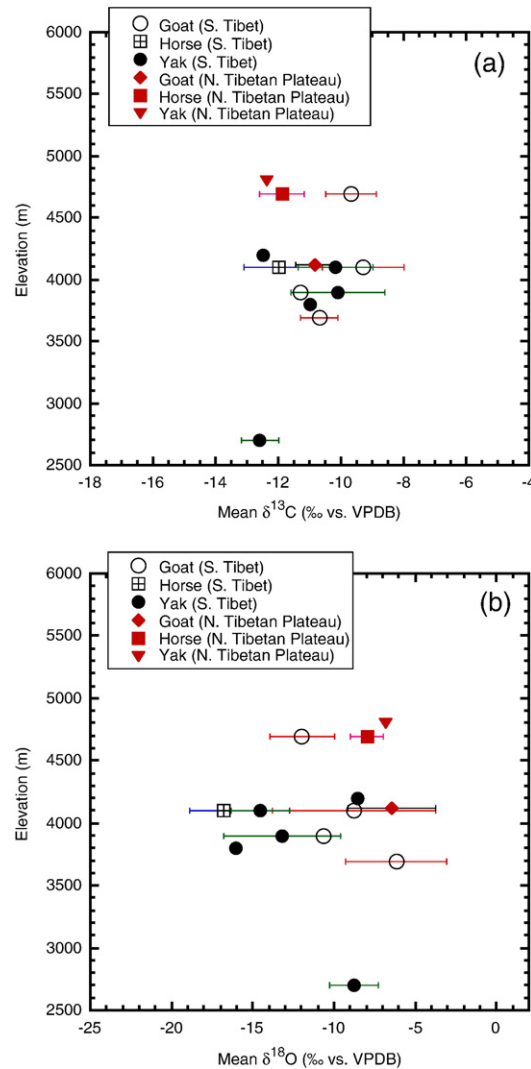


Fig. 7. Variations of the mean  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of modern herbivore tooth enamel with elevation spanning a range from 2700 m to 4800 m a.s.l. on the Tibetan Plateau. Error bars correspond to  $1\sigma$  error on the mean  $\delta^{18}\text{O}$  of all samples from the same species at a given elevation.

values (Fig. 8b), but becomes much weaker when goats are included in regression. A linear regression of individual enamel- $\delta^{18}\text{O}$  values on water- $\delta^{18}\text{O}$  yielded the following equations:

$$\text{Yak and horse : } \delta^{18}\text{O}_{\text{enamel-CO}_3} = 0.8939 \delta^{18}\text{O}_{\text{water}} + 1.3259 \quad (R^2 = 0.6790, N = 235) \quad (1)$$

$$\text{Yak, horse and goat : } \delta^{18}\text{O}_{\text{enamel-CO}_3} = 0.78956 \delta^{18}\text{O}_{\text{water}} + 0.55816 \quad (R^2 = 0.4246, N = 294) \quad (2)$$

Eq. (1) has a slope significantly different from 0 at over 99.9% confidence, and the slope is insignificantly different from 1.0 at the 99% confidence level, further suggesting that the enamel is reflecting local water  $\delta^{18}\text{O}$  values in an almost 1:1 ratio.

In comparison, a linear regression of mean enamel- $\delta^{18}\text{O}$  values on water- $\delta^{18}\text{O}$  yielded the following equation:

$$\text{Yak and horse : } \delta^{18}\text{O}_{\text{enamel-CO}_3} = 1.0471 \delta^{18}\text{O}_{\text{water}} + 3.5104 \quad (R^2 = 0.9277, N = 8) \quad (3)$$

The above equations are different from the relationships previously established for horse (Bryant et al., 1994; Delgado Huertas et al., 1995), goat (Delgado Huertas et al., 1995), cattle (D'Angela and Longinelli, 1990) and bison (Hoppe, 2006). Differences among empirical equations based on different data sets are likely linked to the response of surface water and plant (food source) isotopic composition to humidity as well as the differences in physiology and diet/drinking behavior of different animals (Kohn and Cerling, 2002). However, the enamel-water  $\delta^{18}\text{O}$  relationship based on our data from yaks and horses from the Tibetan Plateau is remarkably similar to that given in Kohn and Cerling (2002) for water-dependent vertebrates (Fig. 8). The strong correlation between

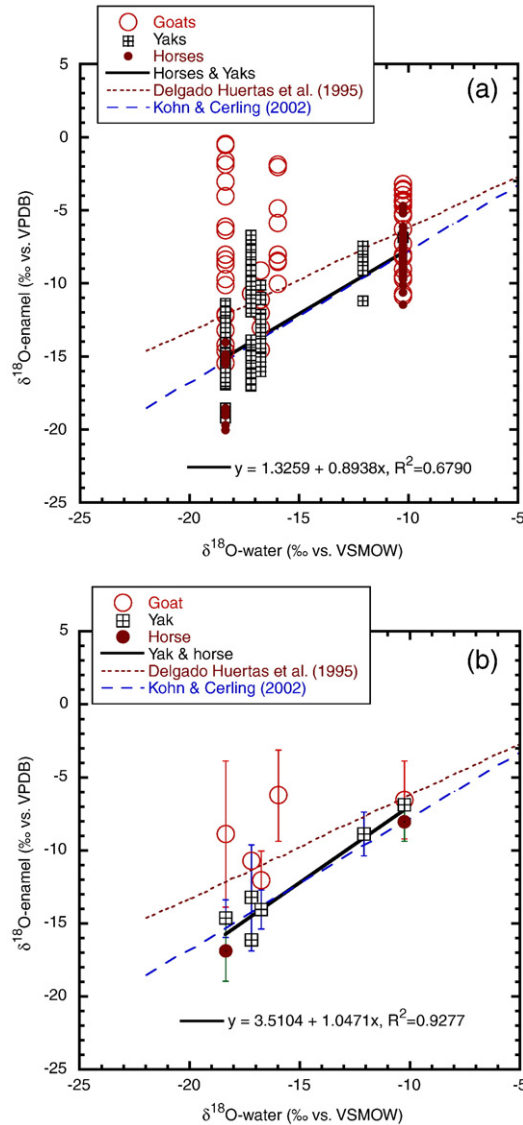


Fig. 8. Plots of (a) enamel– $\delta^{18}\text{O}$  values for goats, yaks and horses vs. water  $\delta^{18}\text{O}$  values, and (b) mean enamel– $\delta^{18}\text{O}$  values for goats, yaks and horses vs. water  $\delta^{18}\text{O}$  values. Solid line is linear regression of enamel  $\delta^{18}\text{O}$  data from both yak and horse. Dashed line is the relationship for vertebrates given in Kohn and Cerling (2002). Dotted line represents the empirical relationship previously established for horses (Delgado Huertas et al., 1995). Error bars in (b) correspond to  $1\sigma$  error on the mean  $\delta^{18}\text{O}$  of all samples from the same species at a given elevation.

enamel– $\delta^{18}\text{O}$  and water– $\delta^{18}\text{O}$  observed in our samples from the Tibetan Plateau confirms that  $\delta^{18}\text{O}$  values of biominerals from large animals that are obligate drinkers generally track the  $\delta^{18}\text{O}$  values of local water (Ayliffe et al., 1992; Bryant and Froelich, 1995; Delgado Huertas et al., 1995; Hoppe et al., 2004).

The climate in the Tibetan region is dominated by the Indian Monsoon and East Asian Monsoon in the summer and by westerly cyclonic activity in the winter (Araguas-Araguas et al., 1998; Thompson et al., 2000). Most of the annual precipitation on the Tibetan Plateau falls during the summer monsoon season (June through August) (Araguas-Araguas et al., 1998). The Indian and East Asian summer monsoons are known to differ substantially with respect to the initial isotopic composition of atmospheric moisture entering the continent and are a significant factor influencing the  $\delta^{18}\text{O}$  of meteoric water in the region (Araguas-Araguas et al., 1998; Johnson and Ingram, 2004; Vuille et al., 2005). During the summer monsoons, moisture bearing winds move from the Indian Ocean and the Pacific Ocean over the continent, and the Tibetan Plateau intensifies the uplift and cooling of the air masses, leading to increased precipitation over the continent. Because condensation preferentially removes heavy isotopes from vapor, the remaining vapor in an air mass and the precipitation formed subsequently becomes more  $^{18}\text{O}$ -depleted as the air mass moves away from its source area. Areas with heavy rainfall can undergo considerable removal of  $^{18}\text{O}$  from the vapor. This “amount effect” (Dansgaard, 1964) or “rain-out effect” dramatically reduces the  $\delta^{18}\text{O}$  of precipitation.

Although there is a strong positive correlation ( $\sim 0.58\text{‰}/\text{°C}$ ) between precipitation  $\delta^{18}\text{O}$  and surface temperature at mid- to high-latitudes, this correlation is weaker or non-existent in low latitudes and in the Asian monsoon region (Rozanski et al., 1993; Johnson

and Ingram, 2004; Vuille et al., 2005). At the IAEA-GNIP stations within and around the Tibetan Plateau, precipitation  $\delta^{18}\text{O}$  values display large seasonal variations and are negatively correlated with the amount of precipitation (Fig. 9). The largest amplitudes of intra-tooth  $\delta^{18}\text{O}$  variations recorded in the modern herbivore teeth analyzed in this study are  $\sim 6\text{‰}$  for yaks,  $\sim 4\text{‰}$  for horses, and  $\sim 9\text{‰}$  for goats, which are smaller than the amplitude of the seasonal  $\delta^{18}\text{O}$  variation of precipitation in Lhasa (Fig. 9). Clearly, the seasonal variations in precipitation  $\delta^{18}\text{O}$  are recorded in the modern herbivore teeth analyzed here. However, these samples were collected from different locations/elevations where seasonal precipitation data are lacking. Therefore, it is presently impossible to assess if (and to what extent) the amplitude of seasonal variations in the  $\delta^{18}\text{O}$  of meteoric water might be reduced or amplified in tooth enamel  $\delta^{18}\text{O}$  record due to dietary/drinking and/or migration behavior of an animal (Kohn, 1996), or due to the time-averaging effect induced by our sampling technique (Passey and Cerling, 2002).

#### 5.4. Implications for paleoclimate and paleoelevation reconstruction

The results from this study have important implications for paleoclimate and paleoelevation reconstructions using C and O isotopic compositions of tooth enamel and other C- and O-bearing substrates. The modern environment on the Tibetan Plateau is mostly water-stressed except the southern slopes of the Himalayas. As a result of high water stress and low atmospheric  $p\text{CO}_2$ , C3 plants in this region typically have  $\delta^{13}\text{C}$  values higher than the average value of  $-27\text{‰}$  for C3 plants. This water/ $\text{CO}_2$ -stress-induced  $^{13}\text{C}$ -enrichment is recorded in the tooth enamel of modern herbivores, and therefore need to be taken into account when interpreting fossil enamel  $\delta^{13}\text{C}$  values in this region. The data presented in this study show that the conservative “cut-off” enamel- $\delta^{13}\text{C}$  value for a pure C3 diet within the Tibetan Plateau should be  $-8\text{‰}$  for modern herbivores and  $-7\text{‰}$  (or even  $-6.5\text{‰}$ ) for fossils if the Tibetan Plateau was in the past as arid as today because the  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  has been lowered by about  $1.5\text{‰}$  since the industrial revolution due to addition of  $^{13}\text{C}$ -depleted  $\text{CO}_2$  from burning of fossil fuels (Marino and McElroy, 1991; Trolier et al., 1996). Serial isotopic analysis of individual teeth can help determine if a high  $\delta^{13}\text{C}$  value is caused by ingestion of a small amount of C4 plants or consumption of C3 plants experiencing water and/or  $\text{CO}_2$  stress. If C4 grasses were consumed by an animal, serial enamel samples from an individual tooth should show significant intra-tooth  $\delta^{13}\text{C}$  variations with higher  $\delta^{13}\text{C}$  values corresponding to summer months and lower  $\delta^{13}\text{C}$  values in enamel formed in winter months. Lack of significant seasonal  $\delta^{13}\text{C}$  variations within individual teeth would rule out the possibility of C4 consumption by the animals.

The  $\delta^{18}\text{O}$  values of tooth enamel from modern yaks and horses within the Tibetan Plateau show a strong positive correlation with the  $\delta^{18}\text{O}$  values of local water (Fig. 8). This is important because it confirms that the  $\delta^{18}\text{O}$  of the meteoric water is the dominant control on the  $\delta^{18}\text{O}$  of tooth enamel (Longinelli, 1984; Luz et al., 1984; Koch et al., 1989; Bryant et al., 1994, 1996) even in water-stressed environments. Therefore, oxygen isotope analysis of tooth enamel is a viable tool in paleoclimate study and can be used as a proxy for the  $\delta^{18}\text{O}$  of precipitation in the Tibetan region. Our results also confirm that enamel–water  $\delta^{18}\text{O}$  relationship is species-

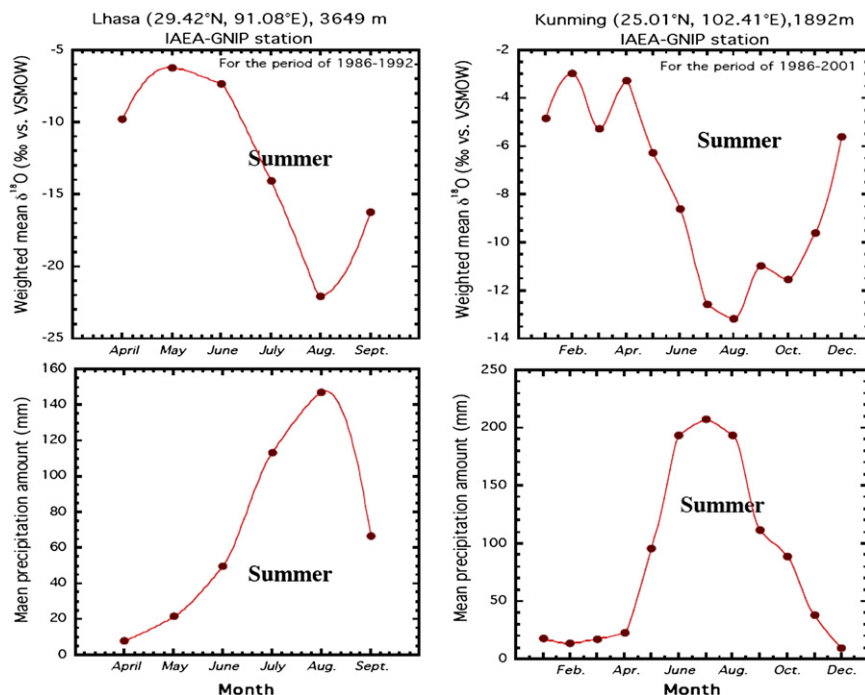


Fig. 9. Weighted mean monthly  $\delta^{18}\text{O}$  values and amounts of precipitation at the IAEA-GNIP stations in Lhasa (left panel) and Kunming (right panel), showing high seasonal variability of the  $\delta^{18}\text{O}$  and the amount of precipitation (IAEA-GNIP database). Both stations are in the Asian monsoon region.

dependent. As such, the use of bulk enamel  $\delta^{18}\text{O}$  data in paleoclimate study without respect to individual species could lead to erroneous interpretations.

The within population  $\delta^{18}\text{O}$  variations for the animals studied here are generally larger than those reported for north American mammals (Clementz and Koch, 2001; Hoppe et al., 2004; Hoppe, 2006). Since the appropriate sample size for reliable estimates of population mean enamel- $\delta^{18}\text{O}$  value depends on the variability of measured enamel- $\delta^{18}\text{O}$  values and the desired level of confidence, the larger intra-population  $\delta^{18}\text{O}$  variations would require a larger sample size in order to reliably estimate the population mean (McClave and Dietrich, 1995; Clementz and Koch, 2001). Assuming enamel- $\delta^{18}\text{O}$  values in a population are normally distributed, we calculated the minimum sample size necessary for reliable estimates of population mean enamel- $\delta^{18}\text{O}$  value to within 0.5‰ to 3‰ at 95% confidence interval using a statistical method (McClave and Dietrich, 1995). As shown in Table 2, goats display the largest intra-population variation of 10.8‰ and therefore a minimum of 28 samples from 28 different individuals are needed in order to establish a robust estimate of the mean enamel- $\delta^{18}\text{O}$  value for goats at a given elevation to within 1‰ with 95% confidence. In comparison, horses show the smallest intra-population variability among the three species studied and require only a minimum of 6 samples (or individuals) in order to provide a reliable estimate of the population mean enamel- $\delta^{18}\text{O}$  value to within 1‰ with 95% confidence (Table 2).

Our data do not show a strong or significant relationship between either the  $\delta^{18}\text{O}$  of water or the  $\delta^{18}\text{O}$  of enamel and the elevation (Figs. 3 and 7). The lack of significant altitude effect on precipitation  $\delta^{18}\text{O}$  in this region has also been noted in other studies (e.g., Tian et al., 2001; Kang et al., 2002). Kang et al. (2002) found a significant negative relationship between  $\delta^{18}\text{O}$  and altitude ( $R^2=0.75$ , significance level 97%) in only one out of four storm events that they sampled. Their data show large temporal variations in precipitation  $\delta^{18}\text{O}$  values ranging from  $\sim -10\text{‰}$  to  $-30\text{‰}$  in the summer monsoon season at each of their study sites in the central Himalayas (Kang et al., 2002). Tian et al. (2001) documented a generally increasing trend in the  $\delta^{18}\text{O}$  values of precipitation and surface water from south to north across the Tibetan Plateau regardless of elevation. The lack of a clear relationship between precipitation  $\delta^{18}\text{O}$  and altitude in this region (Fig. 3) is likely due to natural variations in local weather processes, and in the sources and/or the rain-out history of moisture (Tian et al., 2001; Kang et al., 2002). Changes in the source water temperature and air temperature above the moisture source region also have a significant influence on the initial  $\delta^{18}\text{O}$  of the water vapor and therefore the  $\delta^{18}\text{O}$  of precipitation formed subsequently from the vapor due to the “rain-out” effect and the effect of temperature-dependent fractionation between the liquid and vapor (Jouzel et al., 1997).

Several studies have reported decreasing trends in stream water  $\delta^{18}\text{O}$  with increasing elevation in the Himalayan drainage basins, but the reported lapse rates vary by more than a factor of three, ranging from  $-0.09\text{‰}/100\text{ m}$  to  $-0.29\text{‰}/100\text{ m}$  in catchments lying within a distance of a few hundred kilometers (Ramesh and Sarin, 1992; Bartarya et al., 1995; Garzzone et al., 2000; Dalai et al., 2002). Precipitation  $\delta^{18}\text{O}$  data from the IAEA-GNIP stations in the Asian monsoon region show large seasonal and annual variations. Ice core records from the Tibetan Plateau (Thompson et al., 1989, 1997, 2000) revealed large variations in the precipitation  $\delta^{18}\text{O}$  values over decadal to millennial and longer time scales. For example, the ice core data from Guliya on the Tibetan Plateau show that during the last glacial period between 15,000 and 33,000 years ago, there were about 100  $\delta^{18}\text{O}$  oscillations with amplitudes up to 22‰ and an average period of 200 years (Thompson et al., 1997). The large temporal and spatial variability of precipitation  $\delta^{18}\text{O}$  has been attributed to changes in temperature and the position of the semi-permanent high-pressure system over the Tibetan Plateau (in response to an altered surface thermal regime), the amount effect, the mixing of moist air masses from

Table 2  
Minimum sample size needed for reliable estimates of population mean  $\delta^{18}\text{O}$  with 95% confidence

Species	Largest range of intra-population $\delta^{18}\text{O}$ variation	Desired uncertainty for 95% confidence level (‰)	Minimum sample size
Horse	5.2	0.5	26
	5.2	1	6
	5.2	1.5	3
	5.2	2	2
	5.2	3	1
Yak	8.5	0.5	69
	8.5	1	17
	8.5	1.5	8
	8.5	2	4
	8.5	2.5	3
Goat	8.5	3	2
	10.8	0.5	112
	10.8	1	28
	10.8	1.5	12
	10.8	2	7
	10.8	2.5	4
	10.8	3	3

different sources, complicated weather processes, and variations in monsoon intensity (Thompson et al., 1997; Tian et al., 2001; Kang et al., 2002; Dalai et al., 2002; Blisnuik and Stern, 2005; Vuille et al., 2005).

The lack of strong or significant relationships between the  $\delta^{18}\text{O}$  of modern water and enamel and the elevation in the region, along with the large  $\delta^{18}\text{O}$  variations in precipitation on various time and spatial scales, does not inspire much confidence in quantitative reconstruction of paleoelevation in this region using  $\delta^{18}\text{O}$  values of paleo-meteoric water estimated from fossil enamel and other O-bearing minerals. In a region with complex tectonic history and atmospheric circulation/wind patterns, influences such as changes in atmospheric circulation pattern, temperature, source and rain-out history of atmospheric moisture, mixing of isotopically distinct moisture sources, monsoon strength, and elevation could all have been important in affecting the  $\delta^{18}\text{O}$  of precipitation; and therefore cautions must be exercised in the interpretation of  $\delta^{18}\text{O}$  data in terms of paleoclimate and paleoelevation. Although changes in the  $\delta^{18}\text{O}$  values of authigenic O-bearing minerals in terrestrial environments most likely indicate changes in certain aspects of local climate, it is practically impossible to disentangle the many factors that can have a local influence on the  $\delta^{18}\text{O}$  of precipitation without further constraints from other independent means.

## 6. Conclusions

The stable isotopic compositions of modern herbivore tooth enamel, vegetation, and water were used to examine their relationships with modern environment/elevation of the Tibetan Plateau. The  $\delta^{13}\text{C}$  values of tooth enamel from modern herbivores reflect diets based primarily on C3 plants, consistent with the current dominance of C3 vegetation in the region. Although C4 grasses can be found in the warmest months at high elevations in southern Tibet, they are not a significant dietary component of modern herbivores due to their insignificant presence in local biomass. Our data show that the conservative “cut-off”  $\delta^{13}\text{C}$  value for a pure C3 diet within the Tibetan Plateau should be  $-8\text{‰}$  for modern herbivores and  $-7\text{‰}$  (or even  $-6.5\text{‰}$ ) for fossils if the Tibetan Plateau was in the past as arid as today. Carbon isotopic compositions observed within and between species primarily reflect the C isotopic variations of C3 plants available for consumption in local habitats. Oxygen isotopic compositions of tooth enamel varied widely within and between species. The mean  $\delta^{18}\text{O}$  values of tooth enamel from yaks and horses show a strong positive correlation with water  $\delta^{18}\text{O}$  values, confirming that the  $\delta^{18}\text{O}$  of tooth enamel from large mammals that are obligate drinkers generally tracks the  $\delta^{18}\text{O}$  of meteoric water. Serial isotopic analyses of individual teeth show that intra-tooth  $\delta^{18}\text{O}$  variations primarily reflect seasonal variations in the  $\delta^{18}\text{O}$  of meteoric water. Unfortunately, the  $\delta^{18}\text{O}$  values of modern precipitation and of modern tooth enamel do not show strong correlations with elevation in this region. In light of the large spatial and temporal variability of  $\delta^{18}\text{O}$  values of meteoric water and the lack of a strong relationship between precipitation  $\delta^{18}\text{O}$  and elevation in the Tibetan region, we caution against any quantitative reconstruction of the paleoelevation of the Tibetan Plateau using the  $\delta^{18}\text{O}$  values of paleo-meteoric water estimated from tooth enamel and other O-bearing minerals. Although changes in the  $\delta^{18}\text{O}$  values of authigenic O-bearing minerals in terrestrial environments most likely indicate changes in certain aspects of local climate, teasing apart the many factors (e.g., changes in elevation, temperature, precipitation amount, moisture sources, monsoon strength, etc.) that can have a local influence on the  $\delta^{18}\text{O}$  of meteoric water without other independent constraints is practically impossible in this region with complex tectonic and climatic history.

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