Stable isotope analyses of tooth enamel carbonate of large herbivores from the Tugen Hills deposits: Palaeoenvironmental context of the earliest Kenyan hominids

Damien Roche, Loïc Ségalen, Brigitte Senut, Martin Pickford

To cite this version:


HAL Id: hal-00865276
https://hal.archives-ouvertes.fr/hal-00865276
Submitted on 24 Sep 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Stable isotope analyses of tooth enamel carbonate of large herbivores from the Tugen Hills deposits: palaeoenvironmental context of the earliest Kenyan hominids

Damien Roche\textsuperscript{1,2,*}, Loïc Ségalen\textsuperscript{1,2,*}, Brigitte Senut\textsuperscript{3}, Martin Pickford\textsuperscript{4}

\textsuperscript{1} UPMC Univ Paris 06, UMR 7193 ISTEP, F-75005, Paris, France
\textsuperscript{2} CNRS, UMR 7193 ISTEP, F-75005, Paris, France
\textsuperscript{3} CR2P, UMR 7207 CNRS, MNHN, UPMC, F-75005, Paris, France

Corresponding authors: Damien ROCHE & Loïc SEGALEN
* E-mail addresses: damien.roche@upmc.fr & loic.segalen@upmc.fr

Running head: Stable isotope analyses of herbivore tooth enamel from the Tugen Hills

Keywords: stable isotopes, tooth enamel, Tugen Hills, Neogene, large herbivores, \textit{Orrorin tugenensis}
Abstract

Understanding shifts in past climatic and environmental conditions are crucial for throwing light on human evolution. Available reconstructions of the palaeoecology of faunal and floral assemblages indicate that the earliest Kenyan hominids, including *Orrorin tugenensis*, are associated with forest landscapes. In this study, we present stable isotope data of tooth enamel carbonate of large herbivores associated with these hominids in order further to evaluate their environmental context. Fossil teeth were sampled in the Lukeino Formation (6.1-5.7 Ma, Late Miocene) and the Mabaget Formation (5.3-4.5 Ma, Early Pliocene) at the foot of the Tugen Hills (Great Rift Valley). Despite chemical changes in fossil enamel, preservation of the palaeoenvironmental signals is supported by the difference in δ¹⁸O between hippos and terrestrial fauna. Moreover, the giraffid specimen from the Mabaget Formation exhibits one of the highest δ¹⁸O values, as expected for a drought-tolerant animal. The δ¹³C of large herbivores indicates that the earliest Kenyan hominids inhabited a mixed C₃-C₄ environment as did *Ardipithecus* (Ethiopia) and *Sahelanthropus* (Chad), two early putative hominids. The Tugen Hills might have been a wooded grassland with patches of woodland. There is no record of closed-canopy forest at the time of deposition. We emphasize differences in food habits among herbivore groups. Deinotheres, nearly all elephantids and the giraffid individual had a C₃ diet which reflected browsing behaviour. The intake of C₄ plants prevailed for equids and gomphotheres and was consistent with grazing habits. Most hippos and suids were mixed C₃-C₄ feeders and consequently were generalist herbivores. Bovids and rhinos had a diet ranging from C₃ to C₄ plants. Variations in δ¹³C and δ¹⁸O among faunal assemblages suggest changes in local climate and vegetation. Moister conditions likely occurred in the Early Pliocene and the latest Miocene (ca. 5.7 Ma) than before. The C₃ plant biomass would have increased in the latest Miocene. There were probably denser stands of trees within the depositional environment during the Early Pliocene than in the Late Miocene, which is consistent with palaeontological studies.
1. Introduction

The study of climate and environmental change is crucial for understanding human evolution. It has traditionally been based on the ecological features of faunal and floral assemblages and the depositional context of fossil remains, but recently stable isotope analyses of tooth enamel and pedogenic carbonate have proved useful for exploring African Neogene ecosystems (Cerling et al., 1997, 2011; Kingston and Harrison, 2007; Ségalen et al., 2007; Uno et al., 2011).

Throughout the Neogene, faunas and floras in Africa were influenced by major climatic changes. The asynchronous growth of polar ice caps caused latitudinal shifts of climatic belts and therefore modified continental environments. In the Early Miocene, tropical forest in Africa was widespread on either side of the equator, but with the growth of the Antarctic ice sheet, the tropical belt was displaced northwards and reached the southern part of Europe during the Middle Miocene (Pickford and Morales, 1994; Pickford and Senut, 2003). Later, it shifted southwards to the current position when the Arctic ice cap expanded during the Pliocene (Kennett, 1995; Zachos et al., 2001). The aridification of Africa began in the southwest with the formation of the Namib Desert (17-16 Ma), much earlier than the region of the present-day Sahara (8-7 Ma) (review in Senut et al., 2009). Development of the Great Rift Valley modified atmospheric circulation patterns in East Africa and led to unstable environmental conditions (Coppens, 1983; review in Senut, 2006a). The earliest documented grassland ecosystem is in Uganda some 17.5 Ma ago (Pickford, 2002). Plants which use C₄ pathway emerged around 10 Ma in Kenya (Uno et al., 2011) and expanded in the Plio-Pleistocene when East African environments began going dry (review in Ségalen et al., 2007). Several animal lineages in eastern and northern Africa were replaced by arid-adapted groups immigrating from southern Africa and Eurasia. Other East African lineages withdrew westwards as the tropical forest cover shrank (review in Senut et al., 2009).

Furthermore, hominids appeared in the Late Miocene in Kenya and possibly in Chad and Ethiopia (review in Senut, 2006b, 2011). The origin of bipedalism is a major issue in the study of human evolution as it is one of the markers of our lineage. For decades it was said to have emerged in an open environment, and that the transition from quadrupedalism to bipedality took place in savanna
(savanna hypothesis). However, Pliocene hominids were partly arboreal, but were bipedal when on the ground (review in Senut, 2006b, 2011). Trees were present in the environment (Bonnefille, 2010). The discovery of *Orrorin tugenensis*, a Late Miocene biped, challenged the commonly accepted savanna hypothesis because its postcranial bones indicated that it was a part time tree-dweller (Senut et al., 2001; Pickford et al., 2002; Galik et al., 2004; Gommery and Senut, 2006). Palaeontological studies of the faunal and floral assemblage suggest a well vegetated environment (Pickford and Senut, 2001; Senut, 2006b; Pickford, 2011; Bamford et al., 2013). It was thus important to test these ideas using an alternative approach.

Our study provides a perspective of the environmental context of the earliest Kenyan hominids (including *Orrorin tugenensis*) through stable isotope analyses of teeth of large herbivores associated with the hominid fossils. Tooth remains were collected in deposits in the eastern foot of the Tugen Hills in the Baringo Basin of the Great Rift Valley (figure 1). The sampling localities fall within the Lukeino and Mabaget Formations, dated from the Late Miocene to Early Pliocene (figure 2). Fossil teeth of large herbivores were selected according to their abundance in the deposits and their potential for yielding useful isotopic data. We investigated the stable isotope ratios of the biogenic carbonate in enamel. Carbonate ($CO_3^{2-}$) is incorporated in the phosphate ($PO_4^{3-}$) and hydroxyl (OH) structural sites of biological apatite which is the main constituent of teeth and bones. Bioapatite is a calcium phosphate mineral, the chemical formula of which can be simplified to $Ca_9 (PO_4, CO_3)_3 (OH, CO_3)$ (review in LeGeros and LeGeros, 1984). It is known to record the stable carbon isotope composition of the diet (Krueger and Sullivan, 1984; Cerling and Harris, 1999; Passey et al., 2005) and the stable oxygen isotope composition of the body water which depends on climatic and ecophysiological factors (Longinelli, 1984; Luz and Kolodny, 1985; Kohn et al., 1996). Thus tooth enamel carbonate of wild herbivores can be used to determine aspects of vegetation and climate of their ecosystems.

We analysed tooth enamel rather than bone or dentine because it is more resistant to diagenesis (Lee-Thorp and van der Merwe, 1991; Quade et al., 1992; Ayliffe et al., 1994; Wang and Cerling, 1994; Koch et al., 1997). Compared with bone and dentine, enamel is more mineralized and exhibits a higher crystallinity and a lower porosity (LeGeros and LeGeros, 1984; Asscher et al., 2011). As a result, the recrystallization of bioapatite and the pore-space infilling of secondary minerals are
more limited in enamel than in bone and dentine in the same taphonomic context (Michel et al., 1996; Kohn et al., 1999).

In this paper, we assessed the state of preservation of fossil enamel from the Lukeino and Mabaget Formations before using isotopic data to make palaeoenvironmental interpretations. Fluorine (F), iron (Fe) and manganese (Mn) concentrations were measured in fossil specimens. They are trace elements in modern teeth and bones (LeGeros and LeGeros, 1984; Elliott, 2002; Skinner, 2005). Diagenetic F, Fe and Mn enrichment was observed in herbivore enamel from other Miocene-Pliocene deposits in the Kenyan Rift Valley (Kohn et al., 1999; Schoeninger et al., 2003a; Passey et al., 2007). It is reported to result from pore-space infilling by Fe-Mn oxyhydroxides and dissolution/recrystallization processes of bioapatite in which F replaces OH. We verified that chemical alteration of fossil specimens had no significant effect on palaeoenvironmental signals of biogenic carbonate.

Based on the $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$), we propose a dietary reconstruction of large herbivores associated with the earliest Kenyan hominids from which we build a picture of the local vegetation. Study of variation in $^{18}\text{O}/^{16}\text{O}$ ratio ($\delta^{18}\text{O}$) was aimed at exploring climate change in the Tugen Hills from the Late Miocene to the Early Pliocene. Lastly we compared our stable carbon isotope data with those obtained from herbivore faunas associated with early putative hominids in Chad and Ethiopia.

2. Background

2.1 Geological context

The Lukeino Formation is of Late Miocene age (Bishop et al., 1971; Pickford, 1975, Pickford and Senut, 2001). Magnetochronology and radiometric dating yielded ages from 6.1 to 5.7 Ma (Hill et al., 1985; Deino et al., 2002; Sawada et al., 2002). This formation is about 100 m thick. It overlies the Kabarnet Trachyte Formation and is covered by the Kaparaina Basalt Formation (figure 2). The sediments are fluvio-lacustrine and consist mostly of sandstones, siltstones, tuffs and diatomites. The Kapsomin Basalt and the Rormuch Sills (trachyte intrusions) are points of reference within the Lukeino
Formation, and enable us to distinguish three units: the Kapgoywa Member (ca. 6.1-5.8 Ma), the Kapsomin Member (ca. 5.8 Ma) and the Kapcheberek Member (ca. 5.7 Ma). Remains of the hominid Orrorin tugenensis were found in all three members (Pickford, 1975; Senut et al., 2001; Sawada et al., 2002).

The Mabaget Formation is of Early Pliocene age (Pickford et al., 1983, 2004; Hill, 1985) and overlies the Kaparaina Basalt Formation (figure 2). Its thickness varies throughout the basin. For instance, there are about 150 m of sediments exposed at Pelion (type section) and 40 m at Mosionin. Deposits are mainly sandstones, siltstones and tuffs, deposited in a fluvio-lacustrine system. Radiometric dating provided ages from 5.3 to 3.8 Ma within the Mabaget Formation at Tabarin (Deino et al., 2002). However, a minimum age of ca. 4.5 Ma would fit with the faunal evidence (Pickford et al., 1983, 2004). This formation yielded remains of an indeterminate hominid, possibly Australopithecus praegens or Ardipithecus ramidus (Ferguson, 1989; Pickford et al., 2004). A hominid mandible discovered at Tabarin (Hill, 1985) was dated at 4.5-4.4 Ma by Deino et al. (2002).

2.2 Stable carbon isotopes

For wild large herbivores, bioapatite increases in $\delta^{13}$C by 12-14‰ relative to plants that they usually eat (Lee-Thorp et al., 1989; Cerling and Harris, 1999). Terrestrial plants have differences in $\delta^{13}$C which are related to the photosynthetic pathways, known as C$_3$, C$_4$ and Crassulacean Acid Metabolism (CAM) (Bender, 1971; Deines, 1980). In East African environments, $\delta^{13}$C values range from -35 to -23‰ for C$_3$ plants, and vary between -15 and -11‰ for C$_4$ plants. CAM plants have intermediate values between C$_3$ and C$_4$ plants (Koch et al., 1991; Cerling and Harris, 1999; Cerling et al., 2003c). Trees and most shrubs and forbs use C$_3$ pathway. The majority of East African grasses are C$_4$ plants, except for those growing at high altitude, above 2500-3000 m (Tieszen et al., 1979; Livingstone and Clayton, 1980). C$_4$ plants are better adapted than C$_3$ plants to low atmospheric CO$_2$ concentrations (<500 ppmv), high temperatures (>30°C), high irradiance levels and prolonged water stress (Ehleringer, 1979; Ehleringer et al., 1991, 1997). Plants which use CAM pathway are mainly succulents and favour arid environments due to their high water-use efficiency (Ehleringer, 1979).
The $\delta^{13}C$ of terrestrial plants is controlled by that of CO$_2$ from which they form (Farquhar et al., 1989). The isotopic composition of atmospheric CO$_2$ ($\delta^{13}C_{\text{atm}}$) has been decreasing since the industrial revolution (1800-1850 AD), due to the combustion of $^{13}$C depleted fossil fuels. The pre-industrial $\delta^{13}C_{\text{atm}}$ values (from 1000 to 1800 AD), determined from air trapped in ice cores, are around -6.5‰ (Friedli et al., 1986; Francey et al., 1999). The reconstruction of herbivore palaeodiet and palaeovegetation based on the $\delta^{13}C$ of fossil teeth needs to take account of $\delta^{13}C_{\text{atm}}$ values over geological time. Estimates of $\delta^{13}C_{\text{atm}}$ based on benthic foraminifera $\delta^{13}C$ records range between -6.7 and -6.1‰ from the Late Miocene to the Early Pliocene (7-4 Ma) (Levin et al., 2008; Tipple et al., 2010), which is comparable to pre-industrial values.

Besides the isotopic composition of source air, the $\delta^{13}C$ of C$_3$ and C$_4$ plants can be influenced by light levels and water availability (Ehleringer et al., 1986; Ehleringer and Cooper, 1988; Farquhar et al., 1989; Stewart et al., 1995; Buchmann et al., 1996). Variations in $\delta^{13}C$ are also observed within C$_4$ plants due to different photosynthetic subpathways: the nicotinamide adenine dinucleotide-malic enzyme (NAD), the nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP) and the phosphoenolpyruvate carboxykinase (PCK). For instance, isotopic values average -13‰ for NAD and PCK grasses and -12‰ for nearly all NADP grasses in East Africa (Cerling et al., 2003c). Furthermore, C$_3$ plants growing in dense tropical forest exhibit lower $\delta^{13}C$ values than those growing in savanna and bushland. They are subjected to the “canopy effect” which combines the fixation of $^{13}$C depleted CO$_2$ from soil respiration (-25 to -28‰) with the low light levels beneath the canopy (Medina and Minchin, 1980; van der Merwe and Medina, 1991; Jackson et al., 1993). In tropical Africa, the $\delta^{13}C$ mean value of C$_3$ plants is around -27‰ in savanna and bushland whereas it varies from -31 to -34‰ in understory of closed-canopy forest (Cerling et al., 2003c, 2004).

To reconstruct palaeodiets of large herbivores, we used the mixing C$_3$-C$_4$ model established by Cerling et al. (2005). The $\delta^{13}C$ values of herbivore enamel are divided into three dietary categories. The predominantly C$_3$ diet is characterized by values lower than -8‰. The predominantly C$_4$ diet exhibits values higher than -2‰. The mixed C$_3$-C$_4$ diet includes values between -8 and -2‰. CAM plants are not considered in the interpretation of $\delta^{13}C$ values because they are much less common than C$_3$ and C$_4$ plants. They do not make a significant contribution to diets of extant large herbivores in
East Africa, except perhaps for black rhinos (*Diceros bicornis*) in arid and semi-arid environments (Cerling and Harris, 1999).

### 2.3 Stable oxygen isotopes

The $\delta^{18}O$ of bioapatite depends on the temperature and the $\delta^{18}O$ of the fluid from which it precipitates (Longinelli and Nuti, 1973; Kolodny et al., 1983; Amiot et al., 2007). As mammals are homeothermic animals, they show a constant $\delta^{18}O$ offset between body water and PO$_4$ component in bioapatite (Luz and Kolodny, 1985), and between PO$_4$ and CO$_3$ components in bioapatite ($\Delta$=9‰) (review in Martin et al., 2008). The $\delta^{18}O$ of body water of herbivores is chiefly controlled by the $\delta^{18}O$ of ingested water (*i.e.* drinking water, water contained in food) and the physiological mechanisms of water conservation (review in Kohn and Cerling, 2002).

The stable oxygen isotope composition of meteoric water ($\delta^{18}O_{mw}$) depends on temperature, quantity of precipitation and the $\delta^{18}O$ of atmospheric water vapour. Precipitation is depleted in $^{18}O$ from the equator to the poles ("latitude effect"), and with increasing altitude and distance from coastlines (Dansgaard, 1964; Rozanski et al., 1993; Fricke and O'Neil, 1999; Gonfiantini et al., 2001; Poage and Chamberlain, 2001). In the tropics, seasonal variations in $\delta^{18}O_{mw}$ are ascribed to the "amount effect". A negative correlation is observed between isotopic values and rainfall amounts (Dansgaard, 1964; Rozanski et al., 1993, 1996). Moisture in the air is derived primarily from evaporation of the ocean surface but, in some regions, water vapour supplied by evapotranspiration of the land surface and terrestrial plants is a substantial source of precipitation (Brubaker et al., 1993) and can affect the $\delta^{18}O_{mw}$. For instance, transpired moisture from the Sudd and the Congo Basin is likely responsible for the high isotopic values of rainfall in Ethiopia (Levin et al., 2009).

A rise in temperature and a fall in relative humidity increase the rate of evaporation and plant transpiration, and lead to the $^{16}O$ depletion of surface water, soil water and leaf water (Craig and Gordon, 1965; Dongman et al., 1974). Leaf water is depleted in $^{18}O$ relative to soil water from which it is derived (Yakir, 1992; Yakir and Sternberg, 2000). Body water of mammals is also affected by $^{16}O$ depletion due to the loss of water vapour through sweating and panting (Kohn and Cerling, 2002). As...
a result, the PO$_4$ and CO$_3$ components in herbivore bioapatite are expected to decrease in $\delta^{18}$O when
the environment evolves to more humid or colder conditions (« cooler » is a more appropriate word for
tropical regions). Variations in moisture source may also impact the isotopic values of bioapatite.

Two isotopic patterns are commonly observed in bioapatite of large herbivores from East
Africa. The $\delta^{18}$O is lower for hippos than for terrestrial fauna including bovids, elephants, equids,
giraffids, rhinos and suids (Bocherens et al., 1996; Cerling et al., 2003b, 2008). Hippos wallow in the
water during the day and forage for grass and other fodder at night (Estes, 1991; Kingdon, 1997). It is
argued that their amphibious lifestyle restricts the $^{18}$O enrichment of body water by reducing the loss of
water vapour from body cooling. Night-time intake of plants by hippos could also limit this isotopic
enrichment because plant transpiration is reduced during this period (Bocherens et al., 1996;
Clementz et al., 2008). Furthermore, $\delta^{18}$O values of herbivores with different behaviours record
different aspects of the same environment. The obligate-drinking species (e.g. black rhino, elephant,
hippo) track the $\delta^{18}$O$_{mw}$ more faithfully than the drought-tolerant species (e.g. dikdik, giraffe, oryx). The
latter obtain most of their water from leaves and their isotopic values are impacted by changes in
relative humidity. The highest $\delta^{18}$O values are commonly observed in drought-tolerant herbivores at
the ecosystem level (Kohn et al., 1996; Cerling et al., 2003b, Levin et al., 2006).

3. Materials and methods

We studied 181 molars and premolars of large herbivorous mammals from sixteen localities of
the Lukeino and Mabaget Formations (figure 2). Teeth from the three members of the Lukeino
Formation were collected. Fossil specimens are distributed among nine families within three orders:
artiodactyls (bovids, giraffids, hippos and suids), perissodactyls (equids and rhinos) and
proboscideans (deinotheres, elephantids and gomphotheres). Deinotheres and gomphotheres are
extinct groups. Some teeth were identified at the species or genus level: Nyanzachoerus jaegeri (suid)
in the Mabaget Formation, Nyanzachoerus tulotos (suid) in the Lukeino Formation, Anancus
kenyensis (gomphothere), Deinotherium bozasi (deinothere), Eurygnathohippus (formerly Hipparion,
equid) and Hippopotamus “sensu lato” (hippo) in both formations. Updated faunal lists of these
formations were reported by Pickford et al. (2009).
An enamel powder was obtained from each tooth with a drill and a diamond burr. After cleaning the tooth surface, samples were taken along a vertical groove on the crown. Bulk samples cover several months to a few years of biomineralization, depending on the rate of enamel growth in the species (see table 1 in Kohn, 2004) and the size of the tooth remains.

Measurements of F, Fe and Mn concentrations were performed on nineteen specimens from the Lukeino Formation and twenty from the Mabagat Formation. These individuals are bovids (n=7), deinotheres (n=2), elephantids (n=1), equids (n=2), giraffids (n=1), hippos (n=20), rhinos (n=2) and indeterminate proboscideans (n=4). To provide comparative chemical data, we analysed four molars and premolars of extant bovids (goats/sheep) from the Tugen Hills. These teeth were collected in the field or in the vicinity of dwellings. Before performing the analyses, bulk samples were treated with 0.1 M acetic acid for 10 min to remove diagenetic carbonate. They were rinsed with deionized water and then dried at 60-70°C. After that step, samples were dissolved in 30% nitric acid at 100°C for 1h. Some 30 mg of sample were commonly used for these analyses. The fluorine content was analysed with a fluoride ion selective electrode (Mettler Toledo electrode). The iron and manganese concentrations were tested by ICP-AES (Jobin Yvon 2000 spectrometer). Relative uncertainty of measurements was about 5%. Results are presented in Appendix 1, Supplementary Material.

Stable isotope measurements were made on the CO₃ component of all fossil teeth (n=181). Bulk samples were pretreated with 2% bleach for 15 min and 0.1 M acetic acid for 10 min. These two reactions were aimed at removing organic contaminants and diagenetic carbonate, respectively. Enamel powders were rinsed after each treatment and then dried at 60-70°C. Samples weighing 0.8-1.2 mg were reacted with 100% phosphoric acid in single vessels under vacuum at 70°C for 12 min. The resultant CO₂ was analysed by duel-inlet isotope-ratio mass spectrometry (GV Instruments Isoprime spectrometer). The stable oxygen isotope ratio of carbonate in bioapatite was calculated using the temperature-dependent fractionation factor between calcite and CO₂ produced as a result of phosphoric acid digestion (Swart et al., 1991). The $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios were reported in the $\delta$ notation relative to the international standard PDB, in parts per mil, as: $\delta^{13}\text{C} (\%) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1\right] \times 1000$ and $\delta^{18}\text{O} (\%) = \left[\frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} - 1\right] \times 1000$. Corrections were
based on working carbonate standards calibrated to the reference material NBS-19. Analytical precision of $\delta^{13}$C and $\delta^{18}$O was 0.1‰ for repeated measurements of the standards. Results are listed in Appendix 2, Supplementary Material.

Statistics were performed using the R software. We used the Mann-Whitney test (1947) for comparisons between two independent groups. The latter are significantly different if the p-value is lower than the significance level $\alpha$, fixed at 0.05 (5%).

4. Results and discussion

4.1. State of preservation of fossil enamel

All fossil specimens show higher F, Fe and Mn concentrations than extant individuals (table 1). On average, fossil enamel contains about 40 times more fluorine, 35 times more iron and 120 times more manganese than modern enamel. Comparable F, Fe and Mn concentrations were measured in herbivore enamel from other Mio-Pliocene deposits in the Kenyan Rift Valley (Kohn et al., 1999; Schoeninger et al., 2003a; Passey et al., 2007). No significant difference was observed between individuals from the Lukeino Formation ($n=19$) and those from the Mabaget Formation ($n=20$) (p-value=0.59 for $[F]$, 0.43 for $[Fe]$ and 0.73 for $[Mn]$). Moreover, the chemical composition of fossil enamel is not significantly different between bovids ($n=7$) and hippos ($n=20$) (p-value=0.13 for $[F]$, 0.20 for $[Fe]$ and 0.69 for $[Mn]$). The other families were excluded from statistical comparisons due to the low number of analysed specimens (see section 3). Trace element enrichment in fossil enamel reflects the climatic conditions and tectono-volcanic activity of the East African Rift. Tropical weathering of basaltic lava flows (i.e. mafic rocks) could be responsible for large amounts of Fe and Mn in diagenetic fluids. High F concentrations in these fluids probably result from hydrothermal alteration in the Baringo Basin (Renaut et al., 2002; Tarits et al., 2006). At Cheboit and Kapsomin in the Lukeino Formation, there are extensive tufa deposits which accumulated around hot springs (Pickford et al., 2009).
Previous studies emphasized chemical alterations of herbivore enamel from the Lukeino and Mabaget Formations. Cathodoluminescence observations of fossil specimens other than those analysed indicated an incorporation of rare earth elements and manganese into the apatite lattice (Ségalen et al., 2008). Infrared spectroscopy highlighted a loss of structural carbonate and a gain in crystallinity (Roche et al., 2010). In addition, fossil enamel exhibits a higher calcium/phosphorus mass ratio than modern enamel (Roche et al., 2010).

Despite chemical alterations of fossil enamel, no correlation was observed between isotopic ratios of biogenic carbonate and F, Fe and Mn concentrations. The stable carbon and oxygen isotope ratios of fossil specimens fall within the statistical range of extant large herbivores from East Africa (Bocherens et al., 1996; Cerling et al., 2003b; Levin et al., 2008). We measured values from -14.0 to 1.2‰ for δ¹³C, and between -5.8 and 4.9‰ for δ¹⁸O. Preservation of the palaeoenvironmental signals in tooth enamel carbonate of large herbivores from the Lukeino and Mabaget Formations is supported by the fact that the δ¹⁸O values are consistent with the amphibious lifestyle of hippos and the drought tolerance in giraffids. In each formation, hippos are significantly depleted in ¹⁸O compared with terrestrial fauna including bovids, equids, giraffids, proboscideans, rhinos and suids (p-value<0.001) (figure 3). This isotopic difference was expected (see section 2.3). However, all δ¹⁸O values of fossil hippos, except one, are included in the statistical range of terrestrial herbivores, which is not observed in modern counterparts (Bocherens et al., 1996; Cerling et al., 2003b, 2008). A possible explanation for this is that hippos had a lower affinity for water habitat in Mio-Pliocene time than today. In the Lukeino Formation, the δ¹⁸O values average -2.6 ± 1.2‰ for hippos (n=16) and -0.3 ± 2.5‰ for terrestrial herbivores (n=55). The mean values are -2.0 ± 1.3‰ for hippos (n=32) and -0.5 ± 2.2‰ for terrestrial herbivores (n=78) sampled from the Mabaget Formation. The giraffid specimen from this formation exhibits one of the highest δ¹⁸O values (4.4‰), as expected for a drought-tolerant animal. Extant giraffes are non-obligate drinkers (see section 2.3) and commonly have high δ¹⁸O values, up to 11‰ (Cerling et al., 2003b, 2008; Kingston and Harrison, 2007).

Furthermore, the δ¹⁸O values average 0.0 ± 2.5‰ for bovids (n=32), -1.9 ± 1.0‰ for deinotheres (n=8), -3.1 ± 1.9‰ for elephantids (n=8), 2.1 ± 1.9‰ for equids (n=13), 0.2 ± 1.1‰ for gomphotheres (n=8), 0.4 ± 1.5‰ for rhinos (n=25) and -1.4 ± 1.6‰ for suids (n=21) in the Lukeino and
Mabaget Formations. Difference in $\delta^{18}O$ is significant between elephantids and equids which represent the extremes ($p$-value<0.001). This is consistent with isotopic data of modern counterparts (Bocherens et al., 1996; Cerling et al., 2008). We also calculated a mean $\delta^{18}O$ value of -1.5 ± 2.1‰ for unidentified fossil specimens ($n=17$) which are either elephantids or gomphotheres.

4.2. Palaeodiets of large herbivores

The $\delta^{13}C$ values of herbivore enamel indicate the presence of $C_3$ plant eaters, $C_4$ plant consumers and mixed feeders in the Lukeino and Mabaget Formations (figure 4). These dietary categories were previously observed in other Late Miocene and Early Pliocene faunas in East Africa (Cerling et al., 2003a; Levin et al., 2008; White et al., 2009; Uno et al., 2011).

Bovids from the Lukeino and Mabaget Formations exhibit $\delta^{13}C$ values from -14.0 to 0.7‰. There are ten specimens with a $C_3$ diet, nine with a $C_4$ diet and thirteen with a mixed diet. The distribution of isotopic values displays a gap of 5-6‰ between two groups in each formation. One guild comprises $C_3$ plant eaters whereas the second consists of $C_4$ plant consumers and mixed feeders, except for one individual in the Lukeino Formation close to $C_3$ plant eaters ($\delta^{13}C = -7.7‰$). A discontinuous range of $\delta^{13}C$ values for extant bovids is observed in East African environments and results from browsing and grazing behaviours among species (Cerling et al., 2003c).

The giraffid specimen from the Mabaget Formation has a $\delta^{13}C$ value of -13.1‰ which indicates a $C_3$ diet. The latter is observed in extant giraffes (*Giraffa camelopardalis*) and reflects the intake of tree leaves (Cerling and Harris, 1999).

Fossil specimens of *Hippopotamus “sensu lato”* have $\delta^{13}C$ values between -13.1 and 0.2‰. Most individuals (30 out of 48) are mixed feeders. There are nine specimens with a $C_3$ diet and another nine with a $C_4$ diet. Difference in $\delta^{15}C$ is not significant between individuals from the Lukeino Formation ($n=16$) and those from the Mabaget Formation ($n=32$) ($p$-value=0.23). The mean value is -5.3 ± 3.4‰ for all specimens. Two individuals from the Mabaget Formation are clearly separated from the others and exhibit the lowest $\delta^{13}C$ and highest $\delta^{18}O$ values (figure 4a). These ratios would not be
ecologically discrepant because such an isotopic variation among fossil hippos was previously observed in Pliocene deposits in the Turkana Basin, a tectonically controlled lake system in northern Kenya and southern Ethiopia (Schoeninger et al., 2003b). The heterogeneity of stable isotope values for fossil hippos may reflect differences in their habitat (grassland vs. woodland, river vs. lake), as observed in their modern counterparts (Boisserie et al., 2005; Cerling et al., 2008). These isotopic studies also indicate that most extant hippos (*Hippopotamus amphibius*) are mixed feeders with a predominant intake of C<sub>4</sub> plants.

The δ<sup>13</sup>C values of suids from the Lukeino and Mabaget Formations vary between -11.5 and -1.4‰. Most individuals (17 out of 21) are mixed feeders. Three specimens have C<sub>3</sub> diet and one a C<sub>4</sub> diet. Values average -6.6 ± 2.3‰ for *Nyanzachoerus tulotos* (n=8) in the Lukeino Formation, and -4.8 ± 1.9‰ for *Nyanzachoerus jaegeri* (n=13) in the Mabaget Formation. Although the interspecific difference is not significant (p-value=0.10), the highest values (above -4‰) were only observed in *Nyanzachoerus jaegeri* (4 out of 13 individuals). *Nyanzachoerus jaegeri* could have a higher intake of grasses (C<sub>4</sub> plants) than *Nyanzachoerus tulotos*. This interpretation is in agreement with their dental morphology. *Nyanzachoerus tulotos* has brachyodont cheek teeth (*i.e.* low-crowned teeth) whereas *Nyanzachoerus jaegeri* is characterized by hypsodont cheek teeth (*i.e.* high-crowned teeth) (Harris and Cerling, 2002). The latter species is better adapted to the intake of grasses which are very abrasive plants due to their high phytolith content (Piperno, 2006). Published δ<sup>13</sup>C data of nyanzachoeres provide additional support for our palaeodietary reconstruction (Harris and Cerling, 2002; Levin et al., 2008).

Specimens of the equid *Eurygnathohippus* (formerly called *Hipparion*) show δ<sup>13</sup>C values from -4.8 to 0.2‰ for both formations. The majority of individuals (9 out of 13) has a C<sub>4</sub> diet and the remaining specimens are mixed feeders. This result is consistent with the dentition of these fossil equids which have hypsodont cheek teeth reflecting their grazing habits (Bernor et al., 2010). Other isotopic studies also demonstrate the predominant intake of C<sub>4</sub> plants in *Eurygnathohippus* (Levin et al., 2008; White et al., 2009; Uno et al., 2011). No significant difference in δ<sup>13</sup>C was observed between individuals from the Lukeino Formation (n=8) and those from the Mabaget Formation (n=5) (p-value=0.88). The mean value is -1.4 ± 1.5‰ for all specimens.
Rhinos from the Lukeino and Mabaget Formations have $\delta^{13}$C values from -13.7 to -0.8‰.

Among the individuals analysed, there are ten with a C$_3$ diet, four with a C$_4$ diet and eleven with a mixed diet. The range of $\delta^{13}$C values is discontinuous in each formation. For specimens from the Mabaget Formation, a gap was observed between -5.8 and -3.4‰, within the category of mixed feeders. There are two guilds: one toward the C$_3$ diet (n=14), the other toward the C$_4$ diet (n=4). We noticed three groups in the Lukeino Formation: C$_3$ plant eaters ($\delta^{13}$C lower than -11‰, n=2), C$_4$ plant consumers ($\delta^{13}$C higher than -2‰, n=2) and mixed feeders ($\delta^{13}$C between -8 and -5‰, n=3). The heterogeneity of $\delta^{13}$C values for fossil rhinos could reflect interspecific variation in diet.

*Brachypotherium lewisi*, *Ceratotherium praecox*, *Diceros bicornis* and *Diceros cf. pachygnathus* were reported in faunal lists of the Lukeino and Mabaget Formations (Pickford et al., 2009). In Africa today, there are two rhino species with different dietary habits. The black rhino (*Diceros bicornis*) is a browser and consequently a C$_3$ plant eater whereas the white rhino (*Ceratotherium simum*) is a grazer characterized by a C$_4$ diet (Bocherens et al., 1996; Cerling et al., 2003b).

The $\delta^{13}$C values of *Deinotherium bozasi* vary between -12.7 and -11.9‰ for both formations. All specimens have a C$_3$ diet, which is consistent with their dentition. Deinotheres have brachydont and lophodont cheek teeth which are adapted to browsing (Shoshani and Tassy, 1996). They are reported to have maintained a C$_3$ diet throughout the entire record in East Africa (Cerling et al., 1999; Uno et al., 2011). One individual was analysed in the Lukeino Formation. Its $\delta^{13}$C value is included in the statistical range of the seven specimens from the Mabaget Formation. The mean value is -12.2 ± 0.3‰ for all individuals.

For *Anancus kenyensis*, the $\delta^{13}$C values range from -2.1 to 1.2‰. Almost all individuals have a C$_4$ diet (7 out of 8). One specimen from the Lukeino Formation is a mixed feeder, very close to C$_4$ plant eaters. The cheek teeth of *Anancus kenyensis* are brachydont and bunodont with thick enamel and the crown is usually heavily invested by cementum. On the basis of the dental morphology, this gomphothere species was reported to be adapted to the intake of nuts (tough fruits) and/or grasses (abrasive plants) (Shoshani and Tassy, 1996). Isotopic data of *Anancus kenyensis* are in agreement with grazing habits. They cannot be explained by a prevalent intake of nuts which leads to a C$_3$
signature of bioapatite. The predominantly C\textsubscript{4} diet of Anancus is also confirmed by other isotopic studies (Levin et al., 2008; White et al., 2009; Uno et al., 2011). Three individuals were analysed in the Mabaget Formation. Their δ\textsuperscript{13}C values range within those of the five specimens from the Lukeino Formation. The mean value is -0.1 ± 1.1‰ for all individuals.

Elephantids from the Lukeino and Mabaget Formations exhibit δ\textsuperscript{13}C values between -12.4 and -3.2‰. Nearly all specimens are C\textsubscript{3} plant consumers (7 out of 8). One individual from the Mabaget Formation has a mixed diet, rich in C\textsubscript{4} plants. Stable isotope analyses of extant African elephants (Loxodonta) indicate diets dominated by C\textsubscript{3} plants, even though some specimens show evidence for a mixed C\textsubscript{3}-C\textsubscript{4} diet (Cerling et al., 1999).

Another 17 specimens of proboscideans from the two formations were investigated but could not be identified due to the small size of the tooth remains. They are either elephantids or gomphotheres (Anancus kenyensis). Their δ\textsuperscript{13}C values vary from -12.4 to 0.7‰. Values of C\textsubscript{3} plant eaters (n=5) plot apart from those of mixed feeders (n=4) and C\textsubscript{4} plant consumers (n=8). The C\textsubscript{3} diet group is likely composed of elephantids whereas the C\textsubscript{4}/mixed diet guild may comprise gomphotheres and elephantids.

4.3. Past vegetation and climate of the Tugen Hills

The Lukeino Formation mammalian fauna is dominated by impalas and colobus monkeys, and suggests that the environment comprised thicket, woodland and forest (Pickford and Senut, 2001). Remains of water chevrotains, tree civets, fruit bats and lorisids attest to the presence of forest areas (Pickford et al., 2009, Pickford, 2011). Some fossil leaves from this formation are associated with woodland or dry evergreen forest, others suggest a more humid forest (Senut, 2006b; Senut et al., 2010; Bamford et al., 2013). The faunal assemblage of the Mabaget Formation, including water chevrotains and peafowls, suggests that the environment was heavily vegetated (Pickford et al., 2004, 2009).
The $\delta^{13}$C values of fossil large herbivores indicate that the earliest Kenyan hominids inhabited a mixed C$_3$-C$_4$ environment. They are comparable to those of extant large herbivores living in East African savanna and bushland, taking into account the correction for the anthropogenic $^{13}$C depletion of atmospheric CO$_2$ (Bocherens et al., 1996; Cerling et al., 2003b; Levin et al., 2008). By contrast, extant herbivores living in dense tropical forest - C$_3$ ecosystem marked by the "canopy effect" - exhibit much lower isotopic values than those measured (Cerling et al., 2004). However, a minority of fossil specimens (n=17) show $\delta^{13}$C values lower than -12‰ which may reflect C$_3$ diets in wooded areas. Such negative values were observed in bovids (n=5), giraffids (n=1), hippos (n=1), proboscideans (n=8) and rhinos (n=2) from the Mabaget Formation (figure 4a) and the Kapcheberek and Kapsomin Members of the Lukeino Formation (figure 4b,c). Trees did not form a continuous cover in the Tugen Hills during the Late Miocene and Early Pliocene but they could have formed fairly dense stands where water supply was optimal. Given the depositional context of the fossil remains, patches of woodland were probably located around the Lukeino and Mabaget palaeolakes and along the rivers which drained into the lakes (i.e. riparian vegetation).

C$_3$ diets of hippos, rhinos and suids from the Lukeino Formation were only observed in the Kapcheberek Member, the uppermost unit (figure 4b). Nearly all bovid specimens from this member were C$_3$ plant eaters unlike those from the lower stages of the formation (figure 4c,d). These results suggest that there was more C$_3$ vegetation within the Tugen Hills in the latest Miocene (ca. 5.7 Ma) than before (ca. 6.1-5.8 Ma). A greater C$_3$ plant biomass could reflect a higher woody cover (i.e. more trees and shrubs). The lowest values of C$_3$ plant consumers were observed in ten individuals from the Mabaget Formation (-12.5 to -14.0‰) (figure 4a). $\delta^{13}$C values of C$_3$ plants decrease in shaded and watered conditions (Ehleringer et al., 1986; Ehleringer and Cooper, 1988; Farquhar et al., 1989; Stewart et al., 1995). A possible explanation is that the Tugen Hills were more wooded (leading to less light on the ground) and/or more humid during the Early Pliocene (ca. 5.3-4.5 Ma) than in the Late Miocene (ca. 6.1-5.7 Ma). This is consistent with palaeontological studies based on faunal assemblages (Pickford et al., 2004, 2009). Pollen data from sediments in the Gulf of Aden and offshore the Niger delta suggest a tree expansion in tropical Africa from roughly 5.5 to 3.5 Ma (Bonnefille, 2010). A decline of C$_4$ grass biomass in northeastern Africa by 4.3 Ma is indicated by decreased $\delta^{13}$C values of plant leaf wax from marine deposits of the Gulf of Aden (Feakins et al., 2003b).
Moreover, a shift to more negative $\delta^{13}$C values in pedogenic carbonate from Awash and Turkana Basins suggests that peaks of woody vegetation occurred between 3.9 and 3.2 Ma in Northeast Africa (Cerling et al., 2011).

There are fifteen individuals from the Mabaget Formation and eleven from the Kapcheberek Member which show lower $\delta^{18}$O values than those from the lower members of the Lukeino Formation. These values range from -3.3 to -5.8‰ (figure 4a,b) and they were observed in bovids (n=5), hippos (n=7), proboscideans (n=10), rhinos (n=1) and suids (n=3). They could reflect a stronger “amount effect” on $\delta^{18}$O$_{mw}$, implying that moister conditions occurred in the Tugen Hills during the latest Miocene and the Early Pliocene than before. Rainfall patterns in East Africa today are controlled by major air streams (i.e. northeast and southeast monsoons, westerlies), the position of convergence zones (i.e. Intertropical Convergence Zone, Congo Air Boundary), topography and sea surface temperature (SST) fluctuations in the Indian and Atlantic Oceans (Nicholson, 1996). Observational studies emphasize that warmer Indian Ocean SST are currently associated with more rain in East Africa (review in Cane and Molnar, 2001). Such conditions might have occurred in the latest Miocene and the Early Pliocene. Increased moisture would have led to more C$_3$ vegetation in the latest Miocene, as suggested by $\delta^{13}$C values. Specimens from the Mabaget Formation do not exhibit more negative $\delta^{18}$O values than those from the Kapcheberek Member. Local moisture in the Early Pliocene would have been comparable to that of the latest Miocene. This suggests that the shift to lower $\delta^{13}$C values in C$_3$ plant eaters from the Mabaget Formation is a result of vegetation change. Denser stands of trees - where understory grew in lower light conditions - likely appeared within the Tugen Hills in the Early Pliocene. Statistical comparisons between fossil assemblages at the species level are necessary to confirm or refute these hypotheses; a larger dataset of fossil enamel samples, particularly from the Miocene deposits, is required for these comparisons. Furthermore, the aridity index established by Levin et al. (2006) could not have been applied to fossil assemblages. It takes account of enamel $\delta^{18}$O values of giraffid, dikdik and oryx (two bovid taxa) which are evaporation sensitive herbivores. Unfortunately, we had only one giraffid individual (from the Mabaget Formation) and bovid specimens in both formations were too fragmented to be identified at the genus or species level.

4.4. Large herbivores associated with early potential hominids in Chad and Ethiopia
The $\delta^{13}C$ values of fossil large herbivores from the Tugen Hills were compared with those for counterparts associated with early putative hominids: *Ardipithecus kadabba*, *Ardipithecus ramidus* and *Sahelanthropus tchadensis* (figure 5). In these species, the claimed bipedalism - a distinctive trait of the family Hominidae in the study of hominoid evolution - needs to be reassessed because of insufficient evidence (Senut, 2006b, 2011). Isotopic data of herbivore enamel were compiled from Zazzo (2001), Boisserie et al. (2005), Levin et al. (2008) and White et al. (2009).

Remains of *S. tchadensis* were discovered in the Toros Menalla deposits in the Djurab Desert (Chad) and they were dated at 7-6 Ma on the basis of the faunal assemblage (Brunet et al., 2002). Radiometric dating yielded ages between 7.2 and 6.8 Ma for the sediments thought to contain the hominid fossils (Lebatard et al., 2008), but their stratigraphic position is unclear (Beauvilain, 2008). A detailed study of anthracothere remains from Toros Menalla indicated that the deposits probably span the period 10 to 6 Ma (Pickford, 2008). Palaeontological and sedimentological studies suggest that *S. tchadensis* inhabited a perilacustrine vegetation including gallery and islet forests, wooded savanna and grassland close to a sandy desert (Vignaud et al., 2002; Brunet et al., 2004; Schuster et al., 2006).

In the Awash River Valley (Ethiopia), the remains of *A. kadabba* (5.8-5.2 Ma) and *A. ramidus* (4.5-4.3 Ma) were found in the Late Miocene Adu-Asa Formation and the Early Pliocene Sagantole Formation, respectively (White et al., 1994, 1995; Haile-Selassie, 2001; Semaw et al., 2005; Simpson et al., 2007). Palaeontological studies indicate that *Ardipithecus* might have lived in a woodland with forest patches (WoldeGabriel et al., 2001, 2009; Haile-Selassie et al., 2004; White et al., 2009) whereas the $\delta^{13}C$ values for pedogenic carbonate suggest a more open ecosystem (Cerling et al., 2010, 2011).

The $\delta^{13}C$ values for large herbivores range from -11.3 to 2.0‰ in the Toros Menalla sites (n=46), and vary between -15.7 and 3.3‰ in the Adu-Asa and Sagantole Formations (n=286). They indicate that *Sahelanthropus* and *Ardipithecus* lived in mixed C$_3$-C$_4$ environments as did the Mio-Pliocene hominids from the Tugen Hills. Values lower than -12‰ were not observed in large
herbivores from Toros Menalla. One bovid from the Adu-Asa Formation and some deinotheres and
giraffids from the Sagantole Formation show such negative values. These observations suggest that
woodland represented a scarce or patchy cover within these depositional environments. There is no
record of closed-canopy vegetation.

Deinotheres from the Sagantole Formation had a C\textsubscript{3} diet. Their values are close to those of
to coeval specimens from the Mabaget Formation. Nearly all giraffids from the Chadian and Ethiopian
deposits were C\textsubscript{3} plant eaters. One individual from the Sagantole Formation is reported to have had a
C\textsubscript{4} diet (Levin et al., 2008). The intake of C\textsubscript{4} plants prevailed for equids and gomphotheres from the
Toros Menalla sites and the Adu-Asa and Sagantole Formations. Fossil elephantids from the Awash
River Valley had a C\textsubscript{4} or mixed diet whereas those from the Tugen Hills were C\textsubscript{3} plant consumers or
mixed feeders. C\textsubscript{3}, C\textsubscript{4} and mixed diets were observed in bovids, hippos, rhinos and suids from the
Chadian and Ethiopian deposits.

The highest $\delta^{13}$C values of bovids, equids, rhinos and suids are recorded by specimens from
the Sagantole Formation and they emphasize the relevance of the C\textsubscript{4} grass biomass within the
depositional environment. Maximum values are about 1‰ for suids, 2‰ for equids and suids, and 3‰
for bovids. It is worth noting that variations in relative abundance of the NAD, NADP and PCK grasses
within ecosystems may cause slight differences in $\delta^{13}$C among C\textsubscript{4} plant eaters. Herbivore enamel
associated with a diet of NADP grasses is $^{13}$C enriched by about 1‰ compared to that associated with
diet of NAD/PCK grasses (Cerling and Harris, 1999). The latter favour xeric habitat whereas NADP
grasses are more common in mesic habitat (Hattersley, 1992; Cerling et al., 2003c). Water stress and
light levels can also affect $\delta^{13}$C values of C\textsubscript{4} grasses (Buchmann et al., 1996) and consequently the
isotopic composition of herbivore enamel. C\textsubscript{4} grasses generally exhibit the highest $\delta^{13}$C values in
sunny-watered conditions (Buchmann et al., 1996).

A minority of hippos from the Lukeino and Mabaget Formations had a C\textsubscript{3} diet (9 out of 48
specimens). We observed no C\textsubscript{3} plant eating hippos from the Adu-Asa and Sagantole Formations and
only one individual from Toros Menalla is reported to have had a C\textsubscript{3} diet (Boisserie et al., 2005). These
observations suggest that there was a greater C$_3$ plant biomass (i.e. possibly a higher woody cover) near rivers and lakes within the Tugen Hills than in the other depositional environments.

Lastly we notice that Early Pliocene suids (Mabaget and Sagantole Formations) had higher $\delta^{13}$C values than their Late Miocene counterparts (Adu-Asa and Lukeino Formations and Toros Menalla sites). Increased intake of C$_4$ grasses among suids is correlated with the development of hypsodonty during the Plio-Pleistocene (Harris and Cerling, 2002).

5. Conclusion

The aim of this study was to explore the environment of the earliest Kenyan hominids, using the stable carbon and oxygen isotope composition of tooth enamel carbonate of large herbivores. The $\delta^{13}$C values show differences in diet among taxa between 6.1 and 4.5 Ma (figure 6). Deinotheres, giraffids and nearly all elephantids had a C$_3$ diet that reflects a browsing behaviour. Equids and gomphotheres were mainly C$_4$ plant eaters, which indicates grazing habits. Most hippos and suids were mixed C$_3$-C$_4$ feeders and they were therefore generalist herbivores. Bovids and rhinos had a diet which varied from C$_3$ to C$_4$ plants.

The presence of C$_3$ plants (trees, shrubs and forbs) and C$_4$ plants (tropical grasses) in the Tugen Hills during this period indicates a complex of heterogeneous habitats for the fauna including hominids. The palaeontological studies of the faunal assemblages from the Lukeino and Mabaget Formations and the leaf remains from the Lukeino Formation suggested that there was forest at the time of deposition (Pickford and Senut, 2001; Pickford et al., 2004; Senut, 2006b; Pickford, 2011; Bamford et al., 2013). However, the $\delta^{13}$C of herbivore enamel emphasizes the significance of C$_4$ grass biomass in the Tugen Hills, indicating that trees did not form a continuous cover. Isotopic data would fit with a savanna ecosystem (i.e. a wooded grassland as defined by White, 1986). But, fairly low isotopic values (between -12‰ and -14‰) do not exclude the presence of patches of woodland (figure 6). The latter were likely located in perilacustrine and riparian areas, given the depositional context of the fossil remains.
We observed variations in $\delta^{13}$C and $\delta^{18}$O among faunal assemblages which may reflect changes in local climate and vegetation. The $\delta^{18}$O data suggest that the Tugen Hills experienced more humid conditions in the Early Pliocene (ca. 5.3-4.5 Ma) and the latest Miocene (ca. 5.7 Ma) than before (ca. 6.1-5.8 Ma). The $\delta^{13}$C values indicate that the C$_3$ plant biomass might have increased within the depositional environment during the Late Miocene. The Tugen Hills were probably more wooded in the Early Pliocene than before, which is consistent with palaeontological studies (Pickford et al., 2004, 2009). It is worth noting that our study focussed on large herbivores which represent a part of the fauna discovered in the two formations. Integrating other groups (e.g. gastropods, rodents, primates) in the isotopic approach is necessary to refine the environmental context of the earliest Kenyan hominids.

Postcranial studies show that *Orrorin tugenensis* was a bipedal hominid which could also climb trees (Senut et al., 2001; Pickford et al., 2002; Galik et al., 2004; Gommery and Senut, 2006). This suggests that human bipedalism originated in a mixture of grassy and wooded areas. Patches of woodland provide food such as fruits but also the possibility to escape from savanna predators. Variations in altitude, latitude and tectonics played a role in the distribution of diverse ecosystems (Bailey et al., 2011) and ultimately in the distribution of hominoids (including hominids) and locomotor behaviours, which happen to be more diverse than previously expected. Reconstructing detailed environments in which hominids emerged is still a challenge and further geochemical studies need to be performed in late Middle Miocene deposits, a period in which the dichotomy between the great apes and the hominids might have occurred (Arnason et al., 2000; Senut and Pickford, 2004; Langergraber et al., 2012).

**Acknowledgements**

The authors are grateful for funding from the CNRS (GDRI 193, UMR 7207), the Pierre-and-Marie-Curie University (UMR 7193 ISTEP), the Muséum National d’Histoire Naturelle, the Collège de France and the French Ministry of Foreign and European Affairs (Commission of Archaeological Excavations). We thank the Kenyan Ministry of Education, Research and Technology for excavation and export authorizations. We acknowledge the technical assistance of Nathalie Labourdette and...
Marylène Person. We are thankful to Antoine Zazzo for providing access to unpublished data in his PhD thesis and allowing us to use them for this paper. Lastly we appreciate the comments of the two anonymous reviewers which improved the manuscript.

References


Lebatard A.-E., Bourliès D.L., Duringer P., Jolivet M., Braucher R., Carcaillet J., Schuster M.,


**Figure captions**

**Figure 1:** Location of the Tugen Hills deposits (red square).

**Figure 2:** Stratigraphic section of the Lukeino and Mabaget Formations. Radiometric dates and geomagnetic reversal ages are reported in Pickford et al. (1983), Hill et al. (1985), Deino et al. (2002) and Sawada et al. (2002).

**Figure 3:** Box and whisker plots of δ¹⁸O values for hippos and terrestrial herbivores from the Lukeino and Mabaget Formations. The number of specimens is given in brackets for each group. Outliers are shown as filled circles. “MB” = Mabaget Formation (5.3-4.5 Ma), “LK” = Lukeino Formation (6.1-5.7 Ma).

**Figure 4:** δ¹³C and δ¹⁸O values of large herbivores from the Mabaget Formation (a), the Kapcheberek Member of the Lukeino Formation (b), the Kapsomin Member of the Lukeino Formation (c) and the Kapgoywa Member of the Lukeino Formation (d). The number of specimens is given in brackets for each group. “indet. probos.” = indeterminate proboscideans.

**Figure 5:** δ¹³C values of fossil large herbivores from the Tugen Hills (Kenya), the Awash River Valley (Ethiopia) and the Djurab Desert (Chad). The number of specimens is given in brackets for each group. Box and whisker plots are used for groups including at least five individuals, and outliers are shown as filled circles. “MB” = Mabaget Formation (5.3-4.5 Ma), “LK” = Lukeino Formation (6.1-5.7 Ma), “SG” = Sagantole Formation (5.2-3.9 Ma), “AA” = Adu-Asa Formation (6.4-5.2 Ma) and “TM” = Toros Menalla deposits (7-6 Ma). Stable isotope data of Chadian palaeofauna are taken from Zazzo (2001) and Boisserie et al. (2005). Ages of Toros Menalla sites are indicated in Vignaud et al. (2002) and Brunet et al. (2004). Isotopic data of Ethiopian palaeofauna are compiled from Levin et al. (2008) and White et al. (2009). Ages of Adu-Asa and Sagantole Formations are reported in WoldeGabriel et al. (1994, 2001), Renne et al. (1999), Kleinsasser et al. (2008) and Quade et al. (2008).
Figure 6: Dietary reconstruction of large herbivores from the Lukeino and Mabaget Formations, based on $\delta^{13}$C values of tooth enamel. Individuals are classified according to age and families. The unidentified proboscidean specimens are not represented in the figure.

Table captions

Table 1: Summary of fluorine, iron and manganese concentrations in tooth enamel of extant and fossil large herbivores from the Tugen Hills. "Fm." = formation, "SD" = standard deviation.
Figure 1
Figure 2

Siltstone
Sandstone
Diatomite
Tuff
Basalt
Trachyte

Several tuffs at Tabarin
$^{40}$Ar/$^{39}$Ar age: 3.837 Ma (±0.019) to 5.34 Ma (±0.04) (Deino et al., 2002)
K/Ar age: 5.07 Ma (±0.04) (Pickford et al., 1983)

K-Ar age: 5.66 Ma (±0.14) (Sawada et al., 2002)
$^{40}$Ar/$^{39}$Ar age: 5.73 Ma (±0.05) (Deino et al., 2002)

K-Ar age: 5.61 Ma (±0.17) to 5.80 Ma (±0.22) (Sawada et al., 2002)

Geomagnetic reversal age: between 5.83 Ma and 5.89 Ma (Sawada et al., 2002)

Geomagnetic reversal age: between 6.05 Ma and 6.14 Ma (Sawada et al., 2002)
K-Ar age: 6.06 Ma (±0.13) (Hill et al., 1985)

Stratigraphic position of the sampling localities

Karnworor, Kapchebiret, Kapkirwo, Kinego, Magabat, Mosionin, Ngetakwony, Omserich, Pelion, Sagatia, Tabarin

Kapcheberek
Kapsomin
Aragai, Cheboit, Kaimogol

Kaparaina Basalt Formation
Kapcheberek Member
Romuch Sils
Kapsomin Member
Kapsomin Basalt
Kapgoywa Member

Lukeino Formation

10m

Kabarnet Trachyte Formation
**Figure 6**

<table>
<thead>
<tr>
<th>C3 diet ( (^{13}C &lt; -8%) )</th>
<th>Mixed diet ( (-8% \leq ^{13}C \leq -2%) )</th>
<th>C4 diet ( (^{13}C &gt; -2%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Possible C3 diet in wooded areas</strong> ( (-14% \leq ^{13}C &lt; -12%) )</td>
<td><strong>Mixed diet</strong> ( (-8% \leq ^{13}C \leq -2%) )</td>
<td><strong>C4 diet</strong> ( (^{13}C &gt; -2%) )</td>
</tr>
<tr>
<td>Mabaget Formation ( (ca. 5.3-4.5 \text{ Ma}) )</td>
<td>Lukeino Formation, Kapcheberek Member ( (ca. 5.7 \text{ Ma}) )</td>
<td>Lukeino Formation, Kapsomin Member ( (ca. 5.8 \text{ Ma}) )</td>
</tr>
<tr>
<td>Lukeino Formation, Kapgoywa Member ( (ca. 6.1-5.8 \text{ Ma}) )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bovids</th>
<th>Equids</th>
<th>Hippos</th>
<th>Deinotheres</th>
<th>Giraffids</th>
<th>Rhinos</th>
<th>Elephantids</th>
<th>Gomphotheres</th>
<th>Suids</th>
</tr>
</thead>
</table>

Number of analysed specimens for each group: 4
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Modern specimens (n=4)</th>
<th>Specimens from the Mabaget Fm. (n=20)</th>
<th>Specimens from the Lukeino Fm. (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[F] ppm</td>
<td>Range</td>
<td>149 to 334</td>
<td>4 845 to 19 946</td>
</tr>
<tr>
<td></td>
<td>Mean (± SD)</td>
<td>241 (± 67)</td>
<td>9 879 (± 4 274)</td>
</tr>
<tr>
<td>[Fe] ppm</td>
<td>Range</td>
<td>98 to 180</td>
<td>1 069 to 12 909</td>
</tr>
<tr>
<td></td>
<td>Mean (± SD)</td>
<td>125 (± 33)</td>
<td>4 441 (± 3 188)</td>
</tr>
<tr>
<td>[Mn] ppm</td>
<td>Range</td>
<td>10 to 15</td>
<td>226 to 3 008</td>
</tr>
<tr>
<td></td>
<td>Mean (± SD)</td>
<td>11 (± 2)</td>
<td>1 322 (± 820)</td>
</tr>
</tbody>
</table>