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# INTERPRETING CLIMATIC CHANGE DURING THE LATE PLEISTOCENE/EARLY HOLOCENE IN OKLAHOMA BASED ON THE STABLE ISOTOPE AND AMINO ACID COMPOSITION OF FOSSIL BISON BONES

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# INTERPRETING CLIMATIC CHANGE DURING THE LATE PLEISTOCENE/EARLY HOLOCENE IN OKLAHOMA BASED ON THE STABLE ISOTOPE AND AMINO ACID COMPOSITION OF FOSSIL BISON BONES

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## Abstract

The stable isotope values from both the organic (collagen, $\delta^{13}C$, $\delta^{15}N$) and inorganic (hydroxyapatite, $\delta^{13}C$, $\delta^{18}O$) components within fossil bison bones and teeth were used to determine climatic shifts in Oklahoma during the Late Pleistocene/Early Holocene, especially across a period known as the Younger Dryas (12,800 to 11,500 cal B.P.). Globally, the Younger Dryas is a period of cooler, drier climatic conditions. Fossil bison samples were collected from ten different kill sites within Oklahoma and Texas, ranging in age from 13,350 to 1,700 cal B.P. Modern bison samples were collected from a wildlife preserve in Oklahoma for comparison purposes. Because collagen degrades over time, collagen preservation was assessed by comparing the relative amino acid distributions within fossil samples to a modern collagen sample. The amino acid hydroxyproline is unique to collagen, so its presence in fossil collagen is a strong indicator of preservation. Based on the carbon isotope values, the bison diet consisted of primarily $C_3$ grasses during the Younger Dryas, indicating a cooler climate in Oklahoma at this time. Depleted nitrogen values indicate a humid environment within Oklahoma during the Younger Dryas. Depleted oxygen values also confirm a cooler, wetter environment during this period.

# Introduction

*Purpose*

The stable isotope composition of organic (collagen) and inorganic (hydroxyapatite) constituents within bones and tooth enamel of fossil and modern herbivores can provide information concerning diet (e.g., $C_3$ vs. $C_4$ plants) and environmental conditions (e.g., DeNiro and Epstein 1978; Sullivan and Krueger 1981; Lee-Thorp et al. 1989; Cerling et al. 1997; Jahren et al. 1998; Koch 1998; Balasse et al. 1999; Larson et al. 2001; Balasse 2002; Ambrose and Krigbaum 2003; Hoppe et al. 2006; Bernard et al. 2009; Britton et al. 2009; Clementz et al. 2009; Widga et al. 2010; Zazzo et al. 2012). In the present study, we measured the stable isotope composition of protein ($\delta^{13}C$, $\delta^{15}N$) and carbonate within hydroxyapatite ($\delta^{13}C$, $\delta^{18}O$) from bones and teeth of modern and fossil bison from Oklahoma to determine the extent to which the isotope compositions of these components are reliable proxies for diet and/or environmental conditions. The fossil bison are from ancient kill sites in Oklahoma and Texas that include a time interval of intense cold (approximately 12,800 to 11,500 cal B.P.) known as the Younger Dryas. Fossil samples from kill sites slightly older than and younger than the Younger Dryas were also analyzed for comparison. It is hypothesized that if the fossil materials are sufficiently well-preserved (which will be determined by comparison to modern bison proteins), their stable isotope compositions will reflect changes in vegetation associated with this brief shift in climatic conditions.

*Bone and Teeth*

Bones and teeth are composed of organic and inorganic components. Bone is comprised of approximately 65-70% inorganic material and 24-26% organic material.

Tooth enamel contains more than 96% by weight inorganic material and less than 1% organic material. The mineral hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) makes up most of the inorganic component of bone and teeth and contains a small amount of carbonate, while the organic component consists primarily of collagen, a fibrous protein. Tooth enamel has very low porosity, while bone is very porous (Wang and Cerling 1994).

*Bone Collagen*

The organic component of bone is made up of a fibrous protein called collagen. Collagen accounts for 90% of organic material in bone (Hedges et al. 2005). Carbohydrate-derived keto-acids are used by herbivores to synthesize a significant portion of the amino acids that comprise their collagen (Krueger and Sullivan 1984). The most abundant amino acid within collagen is glycine (Hare et al. 1991) but collagen also contains significant amounts of alanine, proline, and hydroxyproline (Brown 1975) (Table 1). Most of the nitrogen within collagen comes from dietary protein (Hedges et al. 2005). Bone collagen has a slow rate of turnover, so it is thought to represent a single growing season signal (Jahren et al. 1998). However, the collagen within fossil samples of pre-Holocene age is often poorly preserved due to diagenetic factors and may not be reliable for isotopic reconstruction of, for example, paleodiet (Wang and Cerling 1994). Conversely, a cold, dry environment is ideal for collagen preservation (Topalov et al. 2012), which could mean that the collagen within the samples from the Younger Dryas would be better preserved.

**Table 1**
Amino acid composition of bovine collagen[a]

| Amino acids | Bovine skin type I collagen (%) |
|---|---|
| Alanine | 10.50 |
| Arginine | 4.80 |
| Aspartic Acid | 4.80 |
| Cysteine | --- |
| Glutamic Acid | 8.01 |
| Glycine | 33.40 |
| Histidine | 0.46 |
| Hydroxyproline | 9.20 |
| Isoleucine | 1.10 |
| Leucine | 2.50 |
| Lysine | 2.50 |
| Methionine | 0.66 |
| Phenylalanine | 1.30 |
| Proline | 12.90 |
| Serine | 3.80 |
| Threonine | 1.70 |
| Tyrosine | 0.47 |
| Valine | 1.90 |

[a] Modified from Jiang (2006).

*Enamel Hydroxyapatite*

The inorganic component of tooth enamel is composed of hydroxyapatite, a calcium phosphate mineral (Lee-Thorp et al. 1989). This component accounts for 90% of the inorganic material (Larson et al. 2001). The carbonate associated with enamel hydroxyapatite is one of the most useful targets for isotopic analysis because it is less susceptible to diagenetic alteration than bone (Balasse 2002 and references therein). Once formed, enamel is not replaced, so the isotopic signatures are retained throughout an organism's life (Balasse 2002). Changes in the carbon and oxygen isotope

compositions of an animal's hydroxyapatite are caused by environmental and behavioral processes reflected in the enamel of developing teeth (Passey and Cerling 2002). The carbon isotopic signature within hydroxyapatite carbonate reflects the food consumed, whereas the oxygen isotopic signature within hydroxyapatite phosphate and carbonate reflects the water consumed (Balasse 2002 and references therein).

*Bone Hydroxyapatite*

Unlike enamel hydroxyapatite, the carbonate associated with bone hydroxyapatite is replaced throughout an animal's life; thus, its carbon isotopic signature is representative of the average carbon consumed during an animal's lifetime (Clementz et al. 2009). However, higher growth and turnover rates during the animal's early life (Hedges et al. 2007) have a heavy influence on these $\delta^{13}C$ values (Clementz et al. 2009). Carbon and oxygen within the carbonate of bone comes from dissolved bicarbonate in the blood, which is precipitated during skeletal development (Krueger and Sullivan 1984). Because of the small hydroxyapatite crystals and soft, porous nature of bones, as compared to tooth enamel, once exchange and fixation of carbonate during diagenesis of bone (e.g., recrystallization) occurs, bone hydroxyapatite becomes unreliable for isotopic analysis (Ambrose and Krigbaum 2003). Bone hydroxyapatite samples of Pleistocene or older age are often diagenetically altered (Ambrose and Krigbaum 2003).

*Diagenesis*

The use of hydroxyapatite carbonate $\delta^{13}C$ analysis in paleodietary reconstruction is now common. However, the proclivity of bone to diagenesis requires careful preparation of carbonate samples to ensure that isotopic signals being obtained are

original (Garvie-Lok et al. 2004). Using both the organic and inorganic phases of bone in isotopic analysis can yield good results with appropriate precautions. In the case of material over a few thousand years old, unless special conditions have preserved collagen (e.g., freezing), analysis of the organic phase of bone is no longer practical due to deterioration. However, dense bone in some anatomical sites (e.g., petrous) may preferentially preserve collagen for more extended periods of time (Bement et al. 2012b). Using the inorganic phase, which is more stable in fossil material, allows dietary analysis of bone over 10,000 years old (Sullivan and Krueger 1981). Up to 50 million year old herbivore teeth have been used in analysis of tooth enamel (Ambrose and Krigbaum 2003 and references therein). Due to the hard, nonporous nature and large, dense hydroxyapatite crystals in tooth enamel, original isotopic signatures are more likely to be retained because of the low susceptibility to diagenetic alteration (Ambrose and Krigbaum 2003 and references therein). If collagen is preserved, the carbon isotopic signatures from both collagen and hydroxyapatite are useful because they reflect different dietary sources (Garvie-Lok et al. 2004). The $\delta^{13}C$ values of collagen represent dietary carbon from proteins, whereas hydroxyapatite $\delta^{13}C$ values represent overall dietary carbon (Ambrose and Krigbaum 2003). In the present study, the preservation of collagen in fossil samples was determined by comparing the relative distribution of amino acids in the modern samples to the fossil samples.

*C, N, and O Isotopes*

Stable carbon isotope values of collagen and hydroxyapatite of bone and tooth enamel reflect the $\delta^{13}C$ values of the plants that herbivores, such as bison, eat. The $\delta^{13}C$ values of these grazing animals reflect the distribution of $C_3$ vs. $C_4$ grasses within an

area. These values can then be correlated to climate, so the $\delta^{13}C$ values from fossil bison grazers can be used as a proxy for reconstructing paleoclimate and paleovegetation (Hoppe et al. 2006).

Stable nitrogen isotope values within the collagen of bones also reflect the $\delta^{15}N$ values of the ingested vegetation. The $\delta^{15}N$ values can also be used to assess the relative atmospheric humidity of an area (Pate and Anson 2008). Higher $\delta^{15}N$ values correspond to more arid environments (Topalov et al. 2012 and references therein). This can be due to loss of urea, which is enriched in $^{14}N$, or related to adaptations for drought tolerance (Koch 1998 and references therein).

Stable oxygen isotope values within the carbonate in hydroxyapatite of tooth enamel reflect the $\delta^{18}O$ values of the water that animals drink (Balasse 2002 and references therein). Assuming hydroxyapatite forms in equilibrium with the oxygen isotopic value of body water within animals, these $\delta^{18}O$ values can be used as a proxy for reconstructing surface and meteoric water paleotemperatures (Koch 1998). The $\delta^{18}O$ values can also be used to reconstruct moisture variations (Nordt 2001). High $\delta^{18}O$ values correspond to warmer, drier climatic periods, while low $\delta^{18}O$ values correspond to cooler, wetter climatic periods (Dansgaard 1964).

*$C_3$ and $C_4$ Plants*

Plants primarily use either a $C_3$ and/or $C_4$ photosynthetic pathway. Trees, shrubs, woody plants, and cool-season grasses use the $C_3$ pathway, whereas warm-season grasses and corn use the $C_4$ pathway. Temperature and precipitation are the main controlling factors in the distribution of these plants. The $C_3$ plants prefer cool, moist conditions, while $C_4$ plants prefer warm, dry conditions (Hare et al. 1991; Larson et al.

2001). The average $\delta^{13}C$ value for $C_3$ plants is -26.5 ‰, ranging from -20 to -35 ‰, whereas the average $\delta^{13}C$ for $C_4$ plants is -12.5 ‰, ranging from -9 to -16 ‰ (Hare et al. 1991). Due to metabolism, the $\delta^{13}C$ value within collagen of an animal's diet becomes enriched by 5-6 ‰ relative to the vegetation consumed, whereas the $\delta^{13}C$ value within hydroxyapatite becomes enriched by 12 ‰ (Larson et al. 2001) (Figure 1).

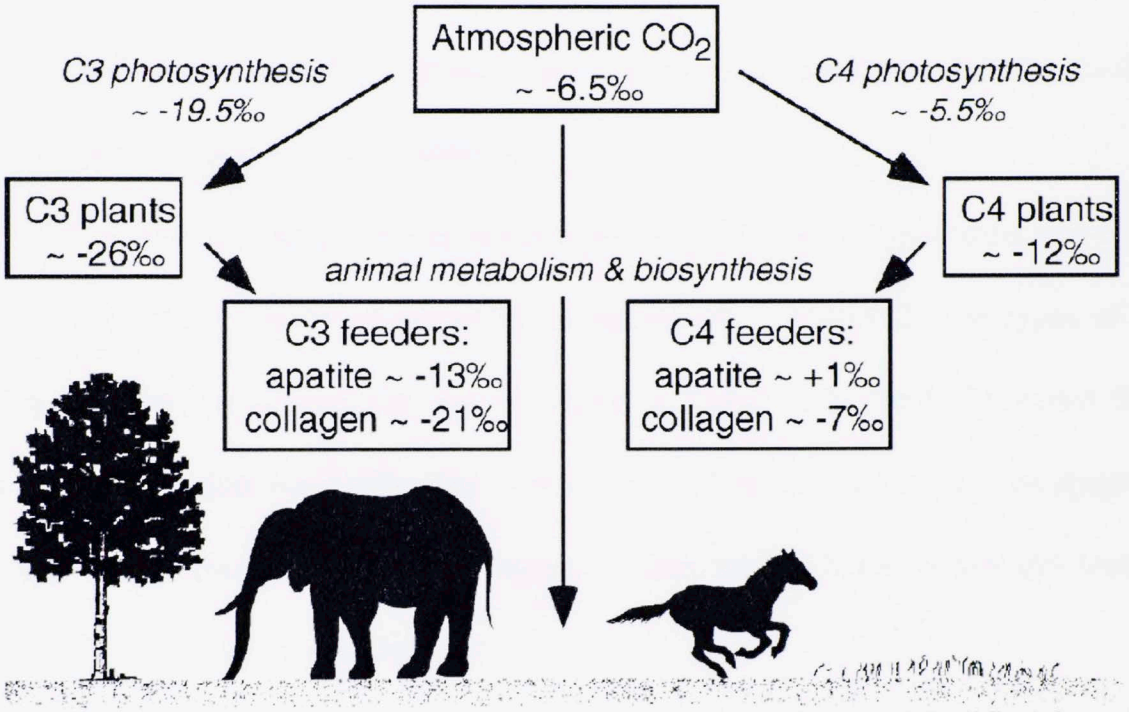


**Figure 1**. Fractionation of carbon through the $C_3$ and $C_4$ pathways. Modified from Koch (1998).

*Younger Dryas*

After the Allerød warming period at the end of the last glaciation, the Younger Dryas, a reversal to a cooler, drier climate, occurred. This lasted from approximately 12,800 cal B.P. until 11,500 cal B.P. The exact timing of this period, as well as the reasons for this abrupt climate shift, has yet to be determined. However, data from the Greenland Icecore Project (GRIP) and Greenland Ice Sheet Project II (GISP2) reveal a distinct shift in climate during the suggested time span (Alley 2000). Possible

hypotheses for the cause of the Younger Dryas include a comet impact, solar radiation, and changes in ocean or atmospheric circulation (Fiedel 2011). The name of this reversal period comes from the Dryas octopetala, an arctic plant that is present in fossil pollen deposits from northern Europe. This cooling period is also marked by an abrupt extinction of large animals. However, bison, because of their large numbers, survived (Haynes, Jr. 2008); thus, there are bones available from this time period for this study.

## Sample Collection

All of the bison samples used in this study were provided by Professor Leland Bement of the Oklahoma Archeological Survey. Modern samples from the same bison specimen included a rib bone and a mandible containing three premolars and three molars, which were collected from the Wichita Mountains Wildlife Refuge near Lawton, Oklahoma. The modern set of samples was used to develop a method for isolating collagen for stable isotope and amino acid analysis and for isolating hydroxyapatite for stable isotope analysis.

Fossil bison samples were obtained from nine kill sites in Oklahoma and one kill site in Texas. The locations of the kill sites are shown in Figure 2. The types of bone samples collected from each kill site are listed in Table 2. It should be noted that in cases where more than one tooth and/or bone were collected at a kill site, the specimens came from more than one animal. Anatomical sites for the bison bones and teeth are shown in Figures 3 and 4, respectively.

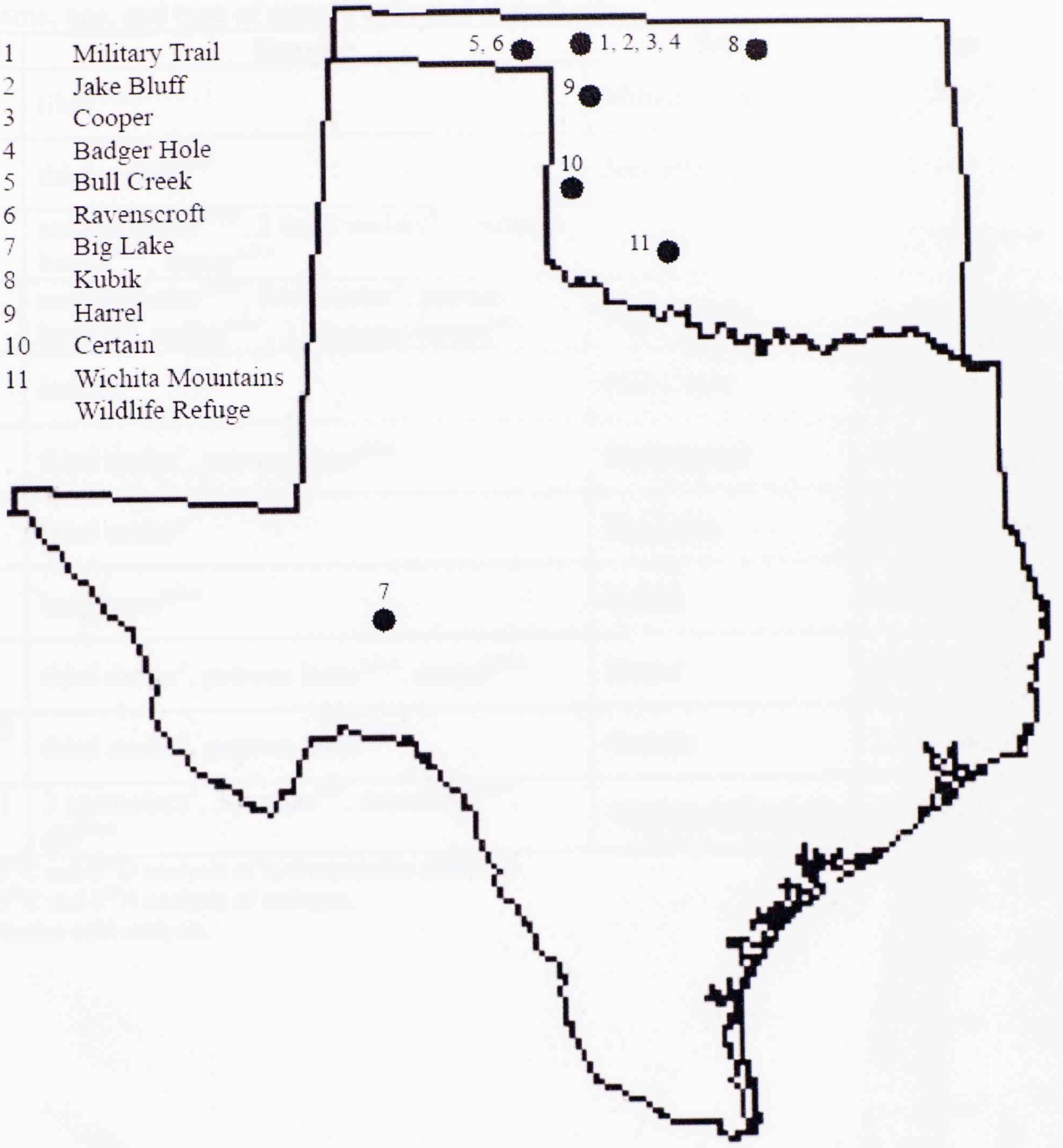


**Figure 2**. Location map of sample sites within Oklahoma and Texas.

**Table 2**

Name, age, and type of sample collected at each site.

| # | Sample | Site | Age |
|---|---|---|---|
| 1 | tibia[a,b,c] | Military Trail | 13,350 cal B.P. |
| 2 | third molar[a,b,c] | Jake Bluff | 12,850 cal B.P. |
| 3 | second molar[a,b,c], 2 third molars[a], 2 petrous bones[a,b,c], femur[a,b,c] | Cooper | 12,550 cal B.P. |
| 4 | second molar[a,b,c], third molar[a], petrous bone[a,b,c], radius[a,b,c], 2 humerus bones[a,b,c] | Badger Hole | 12,350 cal B.P. |
| 5 | incisor[a] | Bull Creek | 10,000 cal B.P. |
| 6 | third molar[a], petrous bone[a,b,c] | Ravenscroft | 10,000 cal B.P. |
| 7 | third molar[a] | Big Lake | 8,900 cal B.P. |
| 8 | long bone[a,b,c] | Kubik | 5,700 cal B.P. |
| 9 | third molar[a], petrous bone[a,b,c], carpal[a,b,c] | Harrel | 2,350 cal B.P. |
| 10 | third molar[a], petrous bone[a,b,c] | Certain | 1,700 cal B.P. |
| 11 | 3 premolars[a], 3 molars[a,b], mandible[a,b,c], rib[a,b,c] | Wichita Mountains | modern |

[a] $\delta^{13}C$ and $\delta^{18}O$ analysis of hydroxyapatite carbonate.

[b] $\delta^{13}C$ and $\delta^{15}N$ analysis of collagen.

[c] Amino acid analysis.

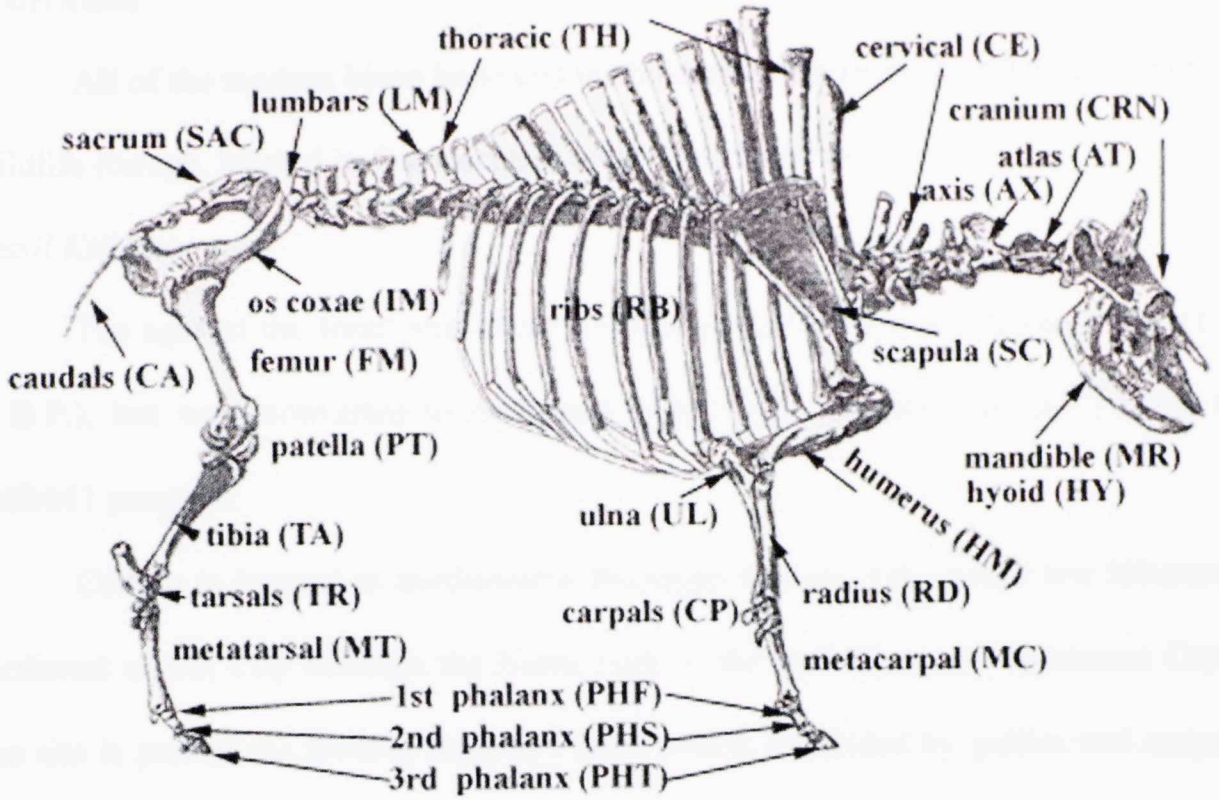


**Figure 3**. Bison skeleton (Todd 2001).

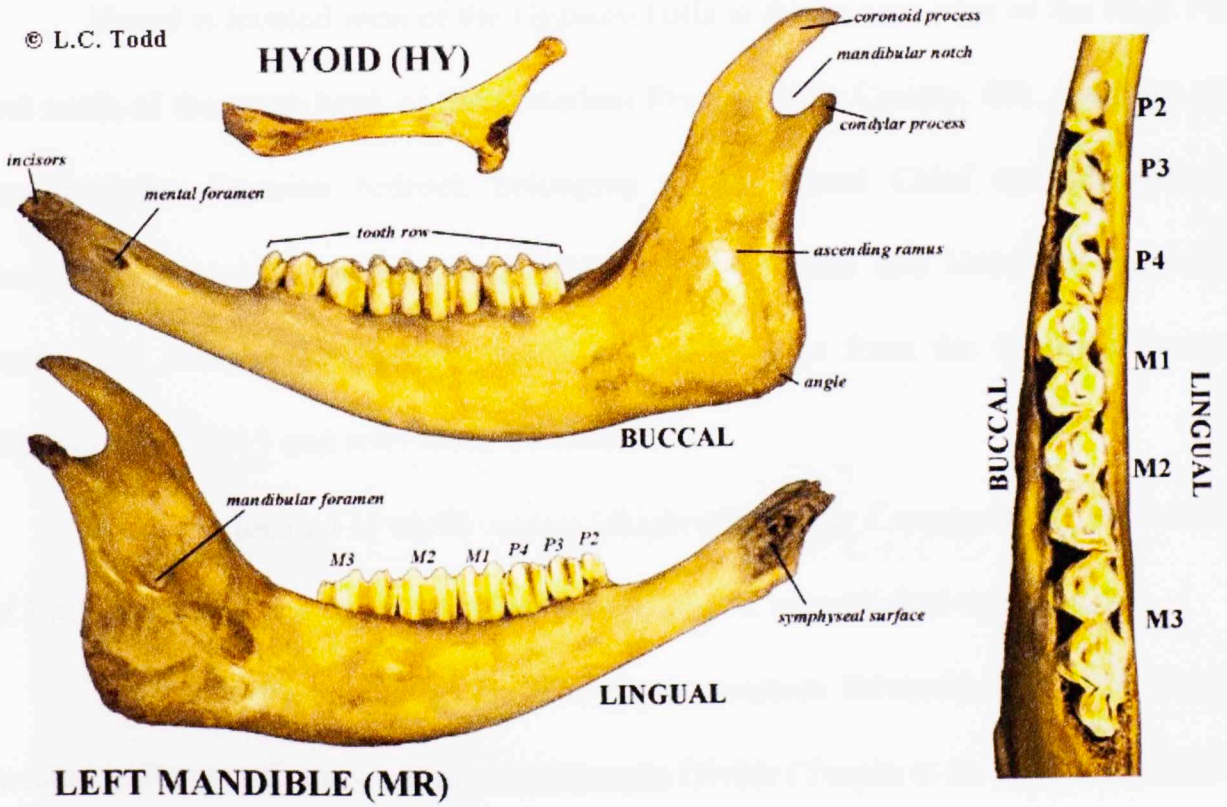


**Figure 4**. Bison mandible (Todd 2001).

*Modern Site*

All of the modern bison bone and teeth samples are from the Wichita Mountains Wildlife Refuge, located in Comanche County, OK, near Lawton.

*Fossil Kill Sites*

The ages of the fossil sites were given in radiocarbon years before present ($^{14}$C yr B.P.), but were converted to calibrated years before present (cal B.P.) using the Calib611 program.

Certain is located in northeastern Beckham County, OK, just a few kilometers northwest of Elk City between the North Fork of the Red River and Sandstone Creek. The site is part of the Rolling Redbed Plains, which is divided by gullies and canyons that have been eroded to expose the kill site. The geology includes Permian sandstone and shale bedrock (Bement and Buehler 2000 and references therein).

Harrel is located west of the Gypsum Hills at the eastern edge of the High Plains and north of the north bank of the Canadian River in Ellis County, OK. Geology of the area includes Permian bedrock belonging to the Cloud Chief and Quartermaster formations, which is superpositioned by late Pleistocene and Holocene wind-blown sands, and overlain by Tertiary sands, silts, and clays from the Ogallala formation (Carlson et al. 2013 and references therein).

Kubik is located in north central Oklahoma in Kay County, along the west side of Little Beaver Creek (Marjorie Duncan, OU, 2014, unpublished manuscript).

Big Lake, the largest saline lake on the western Edwards Plateau of Texas, is located in Reagan County within the Eldorado Divide (Turpin et al. 1997 and references therein).

Ravenscroft is located in western Beaver County, OK, along the left flank of a Bull Creek tributary (Bement et al 2012a). Bull Creek is located 0.5 miles away from the Ravenscroft site along the boundary of the High Plains and Plains Border. Bull Creek is a tributary of the Beaver River that overlies Permian rocks, including Dog Creek Shale, Whitehorse Group, Cloud Chief Formation, and Quartermaster Formation. Rocks in these formations consist of shale and sandstone along with traces of gypsum (Bement et al. 2007 and references therein).

The Badger Hole, Cooper, Jake Bluff, and Military Trail kill sites are all located in Harper County, OK along the Beaver River. These sites are arroyo traps that cut through sandstone bedrock and contain bison remains. Cooper is located 700 meters east of Badger Hole; Jake Bluff is located 300 meters east of Badger Hole and 400 meters west of Cooper; and Military Trail is approximately one mile from Jake Bluff (Bement et al. 2012b) (Figure 5).

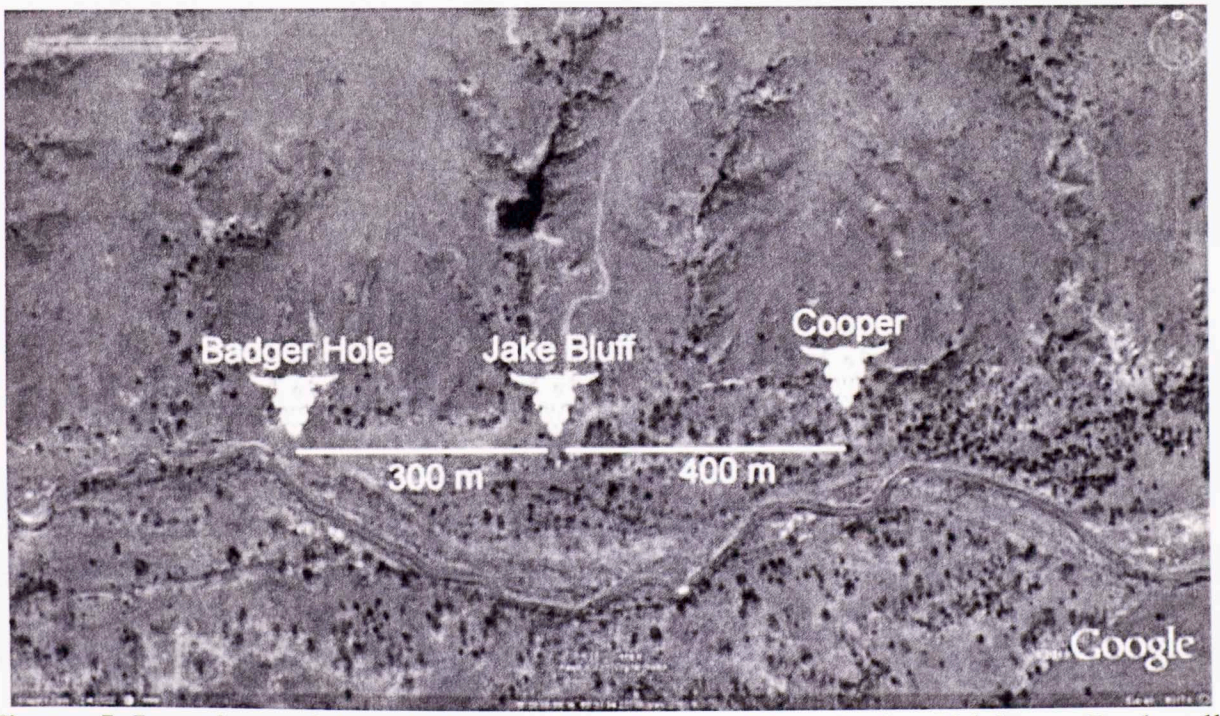


**Figure 5**. Locations of three kill sites along Beaver River in the Oklahoma Panhandle (Bement et al. 2012b).

## Experimental Methods

*Sample Preparation*

Small pieces of the densest parts of bone from the modern and fossil samples were sliced off using a diamond saw. Enamel from modern and fossil teeth samples was removed and separated from dentine using a dental drill. The samples were cleaned in distilled water in an ultrasonic bath and then dried before crushing or powdering with a mortar and pestle.

*Collagen Isolation*

Crushed samples were prepared for collagen isolation using a modified procedure based on the method of DeNiro and Epstein (1978). Approximately 0.5–3.0 g of each sample (Table 1) were treated separately in uncovered 100 mL beakers with 50 mL of 1 M HCl for 20 minutes and then filtered through Whatman glass fiber filters. The filters were placed on aluminum foil and covered with foil to dry overnight.

The dried bone samples were scraped from the filters and placed in separate 250 mL glass beakers. A slightly acidic solution consisting of one drop of 6 M HCl in 50 mL of distilled $H_2O$ was added to each beaker. Each solution was heated (80°C) and continuously stirred with a magnetic stir bar for 10 hours. Next, the solutions were cooled to room temperature and allowed to sit overnight.

Each solution, containing one of the bone samples, was filtered through a Whatman glass fiber filter and rinsed three times with approximately 50 mL of distilled $H_2O$. The filtrate was concentrated on a rotary evaporator down to approximately 2 mL and then transferred to a 4 mL vial and evaporated under $N_2$ over a heating block. The

resulting products, i.e., collagen, were later analyzed for their respective amino acid distributions and stable isotope compositions using the procedures described below.

*Amino Acid Analysis*

Because fossil bone is relatively porous, the potential for collagen to be degraded and/or leached from bone subsequent to burial is a concern (Collins and Riley 2000 and references therein). The C:N values for collagen isolated from fossil bones and/or teeth are often used as an indicator for preservation (e.g., Ambrose 1990). However, it is important to keep in mind that C:N values are of total organic extracts, which may contain materials other than collagen. Another test for the extent to which collagen has been preserved in fossil bone is to compare its amino acid distribution and stereochemistry to that of modern bovine collagen. Amino acid analysis is the most precise way to determine the possible presence of fossil collagen in that there are several amino acids, in particular hydroxyproline, that are specific to collagen and rarely occur in any other types of protein. The following methods were used to determine the amino acid distribution and stereochemistry of collagen isolated from modern and fossil bison bone and teeth samples in this study.

*Hydrolysis*

Approximately 2.5-45.0 mg of each collagen sample was placed in a 25 mL Pyrex tube. Exactly 2 mL of 6 N HCl was added to each tube, sealed under a stream of $N_2$ with a Teflon lined cap, and then heated for 24 hours at 100°C. The hydrolyzates were allowed to cool to room temperature, then transferred to 4 mL vials, and evaporated to dryness under $N_2$.

*Amino Acid Derivatization*

Because amino acids are not volatile, analysis of their distribution and stereochemistry by combined gas chromatography/mass spectrometry (GC/MS) requires that the compounds be derivatized to trifluoroacetyl isopropyl esters.

*Esterification*

Approximately 0.3 mL of isopropanol that was acidified to 3 N by the addition of a stoichiometric amount of acetyl chloride was added to each vial and then sealed under $N_2$ with a Teflon cap. The vials were placed in a heating block at 110°C for one hour. Next, the samples were allowed to cool to room temperature and then were evaporated to dryness under $N_2$. A summary of the esterification step is shown below (Figure 6).

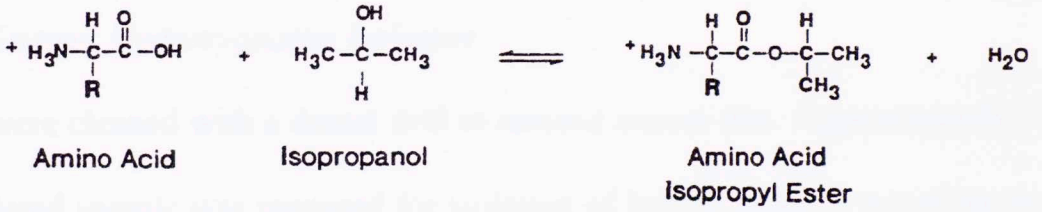


**Figure 6**. Amino acid esterification (Silfer 1991).

*Acylation*

Approximately 0.5 mL of methylene chloride and 0.2 mL of trifluoroacetic anhydride was added to each vial and then sealed with a Teflon cap. The vials were placed in a heating block at 110°C for 10 minutes. The samples were allowed to cool to room temperature and then

were evaporated under $N_2$. A summary of the acylation step is shown below (Figure 7).

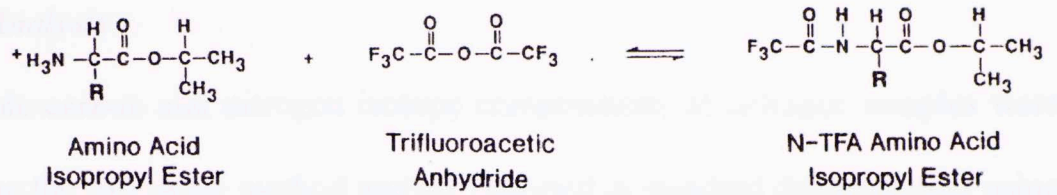


**Figure 7**. Amino acid acylation (Silfer 1991).

*GC/MS Analysis*

Once derivatization was completed, the amino acids were analyzed using a Hewlett Packard GC/MSD equipped with a 50 m x 0.25 mm (i.d.) fused silica capillary column coated with an optically active stationary phase (Chirasil-Val). Approximately 1 mL of dichloromethane was added to each sample and approximately 0.5 µL was injected into the GC/MS to determine amino acid distributions and stereochemistry.

*Bone and Tooth Enamel Hydroxyapatite Isolation*

Samples were cleaned with a dental drill to remove excess dirt. Approximately 5 mg of each powdered sample was prepared for isolation of hydroxyapatite according to a modified version of the method described by Koch et al. (1997). Each bone and tooth sample from both modern and fossil bison was placed in separate glass vials to which a few mL of 2.5% NaOCl were added. The vials were sealed with Teflon-lined caps and allowed to sit overnight at room temperature. This procedure removes any organic matter, such as collagen, that is present in the bone that might interfere with the stable carbon isotope analysis of the hydroxyapatite carbonate. To test the effectiveness and the necessity of using this procedure, a duplicate set of bone samples that were not

treated with NaOCl were analyzed directly for the stable carbon isotope composition of their hydroxyapatite carbonate fractions.

*Stable Isotope Analysis*

The stable carbon and nitrogen isotope compositions of collagen samples were determined using the following method and are reported in standard delta notation using the following equation:

$$\delta_{sample} = (R_{sample} / R_{standard} - 1)\ 1000$$

where R is the ratio of the heavy isotope to the light isotope of the element (e.g., $R = {}^{13}C/{}^{12}C$ for carbon).

For organic carbon isotope analysis, approximately 200-300 µg of collagen was analyzed using a Costech (Valencia, CA) 4010 Elemental Analyzer (EA) that is equipped with a furnace reactor column packed with the reagents chromium oxide (Costech 011001) and silvered cobalt oxide (Costech 011007) in accordance with the Costech 4010 manual at a furnace temperature of 1000°C. The reduction column is packed with copper reduced wire (Costech 011013) at a temperature of 650°C. The GC column is at a temperature of 55°C. In cases where stable carbon and stable nitrogen isotope compositions were simultaneously determined for a sample, the same method was employed with one modification, i.e., the magnet of the mass spectrometer was jump calibrated from $N_2$ to $CO_2$ to allow for duel measurement of carbon and nitrogen isotopes within the same run.

The samples were weighed on a micro-balance and wrapped in tin capsules (Costech 041074) and then placed in sequence in a Costech zero blank autosampler which is mounted on the elemental analyzer. The samples were purged with high purity

helium (99.9999%) to remove air and then analyzed by flash combustion. The resulting sample peak is carried by a helium stream at a flow rate of 100 mL/min to a Thermo Conflo III interface with dilution which is connected to the ion source of a Thermo Delta V Plus isotope ratio mass spectrometer. The $\delta^{13}C$ values are reported relative to the VPDB standard, while the $\delta^{15}N$ values are reported relative to $N_2$ in air.

The stable carbon and oxygen isotope composition of the hydroxyapatite carbonates were determined using the following method:

Approximately 200-300 μg of carbonate was loaded into 12 mL borosilicate vials (Labco 938 W) which were sealed with butyl rubber septa caps. The vials were then placed in a temperature-controlled sample tray heated at 50°C and flushed with ultra high purity He (99.999%) using a Thermo Gas Bench II equipped with a Combi PAL autosampler flushing needle for 360 seconds to remove air. Next, 0.4 mL of 100% phosphoric acid was manually injected into the vials with a syringe and the reaction was allowed to proceed for at least 1.5 hours. The vials were then sampled with the PAL measurement needle and the headspace $CO_2$ was analyzed for $\delta^{13}C$ and $\delta^{18}O$ using a Thermo Delta V Plus isotope ratio mass spectrometer.

The carbon and oxygen isotopic compositions are reported in standard δ-notation:

$$\delta_{sample} = (R_{sample}/R_{standard} - 1)\ 1000\ ‰$$

where R is $^{13}C/^{12}C$ for carbon and $^{18}O/^{16}O$ for oxygen.

The average δ value of 10 sample pulses is expressed relative to VPDB on a scale such that $\delta^{13}C$ and $\delta^{18}O$ of NBS-19 is +1.95 ‰ and -2.20 ‰, respectively.

In cases when comparing previous studies to the present study in the discussion to follow, oxygen values that were reported in VSMOW were converted to VPDB using the equation below:

$$VPDB = (VSMOW - 30.86) / 1.03086$$

## Results

The stable isotopic measurements of collagen ($\delta^{13}C$ and $\delta^{15}N$) and hydroxyapatite ($\delta^{13}C$ and $\delta^{18}O$) from the bones and teeth of modern and fossil bison from each site are summarized in Tables 3 to 12. Graphical representations of these results can be seen in Figures 8 to 13. Chromatograms from the GC/MS analyses of the amino acid distribution of the collagen samples are shown in Figures 14 to 29.

**Table 3**
Isotopic values of collagen and hydroxyapatite for the modern bison bones and teeth.

| | | Collagen | | Hydroxyapatite | | |
|---|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB)[b] | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| Modern | P2 | | | -7.04 | -6.14 | -8.16 |
| | P2 (dup) | | | -7.12 | | |
| | P3 | | | -7.37 | -7.42 | -2.38 |
| | P3 (dup) | | | -7.40 | | |
| | P4 | | | -7.53 | -7.24 | -3.10 |
| | P4 (dup) | | | -7.51 | | |
| | M1 | | | -8.29 | -7.55 | -6.21 |
| | M1 (dup) | | | -8.20 | | |
| | M2 | -18.58 | | -5.10 | -4.84 | -3.42 |
| | M2 (dup) | -18.63 | | -5.17 | | |
| | M2[a] | -18.75 | 4.87 | | | |
| | M2 (dup)[a] | -19.10 | 4.93 | | | |
| | M3 | | | -7.10 | -6.90 | -4.11 |
| | M3 (dup) | | | -7.12 | | |
| | M3[a] | | | | -7.41 | -2.85 |
| | M3 (dup)[a] | | | | -7.30 | -3.40 |
| | Mandible | -17.38 | | -6.69 | -5.73 | -7.36 |
| | Mandible (dup) | -17.59 | | -6.67 | | |
| | Mandible[a] | -17.99 | 3.37 | | | |
| | Mandible (dup)[a] | -18.08 | 3.49 | | | |
| | Rib | -17.27 | | -7.17 | -6.16 | -6.68 |
| | Rib (dup) | -17.53 | | -7.20 | | |
| | Rib[a] | -17.39 | 4.14 | | | |
| | Rib (dup)[a] | -17.30 | 4.37 | | | |

[a] Re-runs of same sample material.
[b] Not treated with 2.5% NaOCl.

**Table 4**
Isotopic values of collagen and hydroxyapatite for samples from the Certain site.

| | | Collagen | | Hydroxyapatite | |
|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **1,700 cal B.P.** | Certain M3 | | | 0.24 | -6.37 |
| | Certain M3 (dup) | | | 0.29 | -6.36 |
| | Certain Petrous | -10.02 | 6.08 | -2.53 | -4.01 |
| | Certain Petrous (dup) | -10.38 | 5.99 | -2.52 | -4.81 |

**Table 5**
Isotopic values of collagen and hydroxyapatite for samples from the Harrel site.

| | | Collagen | | Hydroxyapatite | |
|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **2,350 cal B.P.** | Harrel M3 | | | -0.56 | -6.07 |
| | Harrel M3 (dup) | | | -0.61 | -6.47 |
| | Harrel Petrous | -10.26 | 5.59 | -2.38 | -5.01 |
| | Harrel Petrous (dup) | -10.53 | 5.61 | -2.36 | -4.70 |
| | Harrel Carpal | -17.10 | 3.16 | -2.40 | -6.79 |
| | Harrel Carpal (dup) | | | -2.43 | -6.86 |

**Table 6**
Isotopic values of collagen and hydroxyapatite for the bone sample from the Kubik site.

| | | Collagen | | Hydroxyapatite | |
|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **5,700 cal B.P.** | Kubik Long Bone | -12.24 | 6.69 | -2.26 | -5.19 |
| | Kubik Long Bone (dup) | -12.27 | 6.57 | -2.43 | -7.88 |

**Table 7**
Isotopic values of hydroxyapatite for the tooth sample from the Big Lake site.

| | | Hydroxyapatite | |
|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **8,900 cal B.P.** | Big Lake M3 | -0.35 | -1.48 |
| | Big Lake M3 (dup) | -0.40 | -1.47 |

**Table 8**
Isotopic values of collagen and hydroxyapatite for samples from the Ravenscroft site.

| | | Collagen | | Hydroxyapatite | |
|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **10,000 cal B.P.** | Ravenscroft M3 | | | 0.91 | -3.10 |
| | Ravenscroft M3 (dup) | | | 1.01 | -3.14 |
| | Ravenscroft Petrous | -13.67 | | | |
| | Ravenscroft Petrous (dup) | -10.48 | | | |
| | Ravenscroft Petrous[a] | -10.95 | 10.67 | | |
| | Ravenscroft Petrous[a] | -11.76 | 10.10 | -2.29 | -6.89 |
| | Ravenscroft Petrous (dup)[a] | -10.99 | 10.11 | -2.26 | -5.81 |

[a] Re-runs of same sample material.

**Table 9**
Isotopic values of hydroxyapatite for the tooth sample from the Bull Creek site.

| | | Hydroxyapatite | |
|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **10,000 cal B.P.** | Bull Creek Incisor | -0.63 | -3.00 |
| | Bull Creek Incisor (dup) | -0.63 | -3.14 |

**Table 10**
Isotopic values of collagen and hydroxyapatite for samples within the Younger Dryas.

| | | Collagen | | Hydroxyapatite | |
|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **12,350 cal B.P.** | Badger Hole M2 | -20.47[b] | | -2.86 | -7.03 |
| | Badger Hole M2 (dup) | -19.02[b] | | | |
| | Badger Hole M2[a] | -19.45[b] | 1.6[b] | -2.16 | -5.97 |
| | Badger Hole M2 (dup)[a] | -18.84[b] | 2.09[b] | -2.25 | -6.25 |
| | Badger Hole M3 | | | -2.36 | -5.34 |
| | Badger Hole M3 (dup) | | | -2.36 | -5.32 |
| | Badger Hole Petrous | -23.41[b] | | -3.93 | -8.33 |
| | Badger Hole Petrous (dup) | -21.45[b] | | -3.87 | -9.02 |
| | Badger Hole Petrous[a] | -21.31[b] | -4.3[b] | | |
| | Badger Hole Petrous (dup)[a] | -21.74[b] | -4.14[b] | | |
| | Badger Left Radius | -10.91 | 9.70 | -3.62 | -6.93 |
| | Badger Left Radius (dup) | | | -3.64 | -6.49 |
| | Badger Left Humerus | -12.52 | 9.25 | -3.80 | -6.32 |
| | Badger Left Humerus (dup) | -12.60 | 9.14 | -3.76 | -6.95 |
| | Badger Right Humerus | -14.36 | 4.38 | -3.51 | -7.46 |
| | Badger Right Humerus (dup) | -14.10 | 4.61 | -3.41 | -5.55 |
| **12,550 cal B.P.** | Cooper M2 | -22.18[c] | | -2.85 | -7.09 |
| | Cooper M2 (dup) | -20.81[c] | | | |
| | Cooper M2[a] | -19.92[c] | 1.42[c] | -3.02 | -6.65 |
| | Cooper M2 (dup)[a] | -18.48[c] | 1.8[c] | -3.07 | -6.65 |
| | Cooper M3 | | | -3.35 | -4.73 |
| | Cooper M3 (dup) | | | -3.35 | -4.77 |
| | Cooper M3 2 | | | -0.13 | -2.21 |
| | Cooper M3 2 (dup) | | | -0.10 | -2.14 |
| | Cooper Petrous | -19.11[b] | | -2.66 | -6.95 |
| | Cooper Petrous (dup) | | | -2.71 | -5.28 |
| | Cooper Petrous[a] | -18.63[b] | 3.95[b] | | |
| | Cooper Petrous (dup)[a] | -19.3[b] | 3.88[b] | | |
| | Cooper Petrous 2 | -22.77 | | -3.61 | -6.17 |
| | Cooper Petrous 2 (dup) | -19.00 | | -3.76 | -10.01 |
| | Cooper Petrous 2[a] | -18.94 | 3.24 | | |
| | Cooper Femur | -13.94[b] | 8.05[b] | -1.96 | -6.82 |
| | Cooper Femur (dup) | -13.85[b] | 8.11[b] | -1.97 | -7.27 |

[a] Re-runs of same sample material.
[b] Unreliable values as determined by amino acid analysis.
[c] Values not used in calculated averages that are plotted in following graphs.

**Table 11**
Isotopic values of collagen and hydroxyapatite for the tooth from the Jake Bluff site.

| | | Collagen | | Hydroxyapatite | |
|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **12,850 cal B.P.** | Jake Bluff M3 | ---[a] | ---[a] | -1.31 | -3.39 |

[a] Values could not be determined due to poor collagen preservation as determined by amino acid analysis.

**Table 12**
Isotopic values of collagen and hydroxyapatite for the bone from the Military Trail site.

| | | Collagen | | Hydroxyapatite | |
|---|---|---|---|---|---|
| | Sample | $\delta^{13}C$ ‰ | $\delta^{15}N$ ‰ | $\delta^{13}C$ ‰ (VPDB) | $\delta^{18}O$ ‰ (VPDB, 25°C) |
| **13,350 cal B.P.** | Military Trail Tibia | -9.36[a] | 7.2[a] | -4.30 | -0.90 |
| | Military Trail Tibia (dup) | -9.42[a] | 7.03[a] | -3.90 | 0.02 |

[a] Unreliable values as determined by amino acid analysis.

*Stable Isotope Analysis of Bone Collagen*

The average of the $\delta^{13}C$ values from the well-preserved bone collagen samples from the Cooper site (12,550 cal B.P.) is -20.24 ± 2.19. The $\delta^{13}C$ values become enriched in $^{13}C$ until 10,000 cal B.P., then gradually become depleted in $^{13}C$ until 2,350 cal B.P. (Figure 8). The values increase to a maximum average of -10.20 ± 0.25 at 1,700 cal B.P. and then become depleted in $^{13}C$ at the present. The average values of just the petrous bone samples follow a similar pattern (Figure 8).

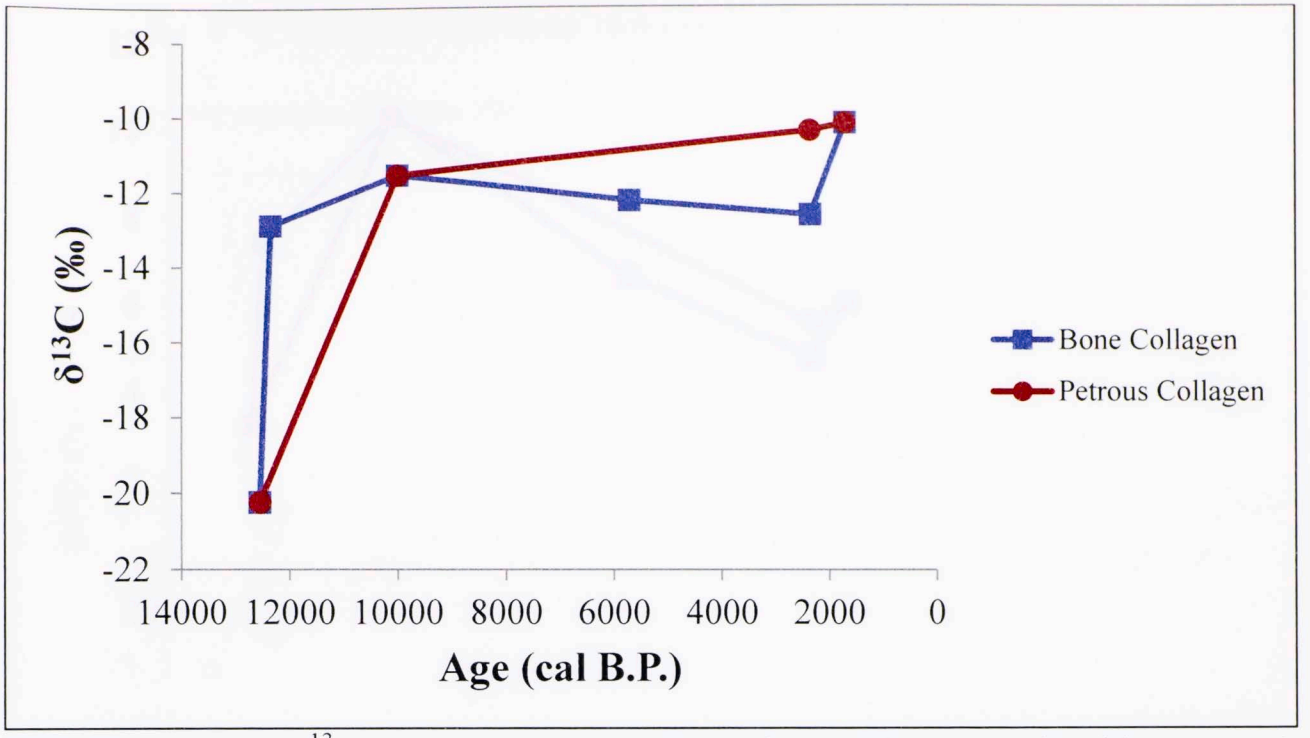


**Figure 8**. Average $\delta^{13}C$ values per site of collagen from all bone samples (blue squares) and just petrous samples (red circles), excluding values determined unreliable by amino acid analysis.

The $\delta^{15}N$ value for the well-preserved bone collagen sample from the Cooper site is 3.24. The average $\delta^{15}N$ values follow the same pattern of enrichment and depletion as the $\delta^{13}C$ values, but have a maximum average of $10.29 \pm 0.33$ at 10,000 cal B.P. The average values of just the petrous bone samples also show the same pattern (Figure 9).

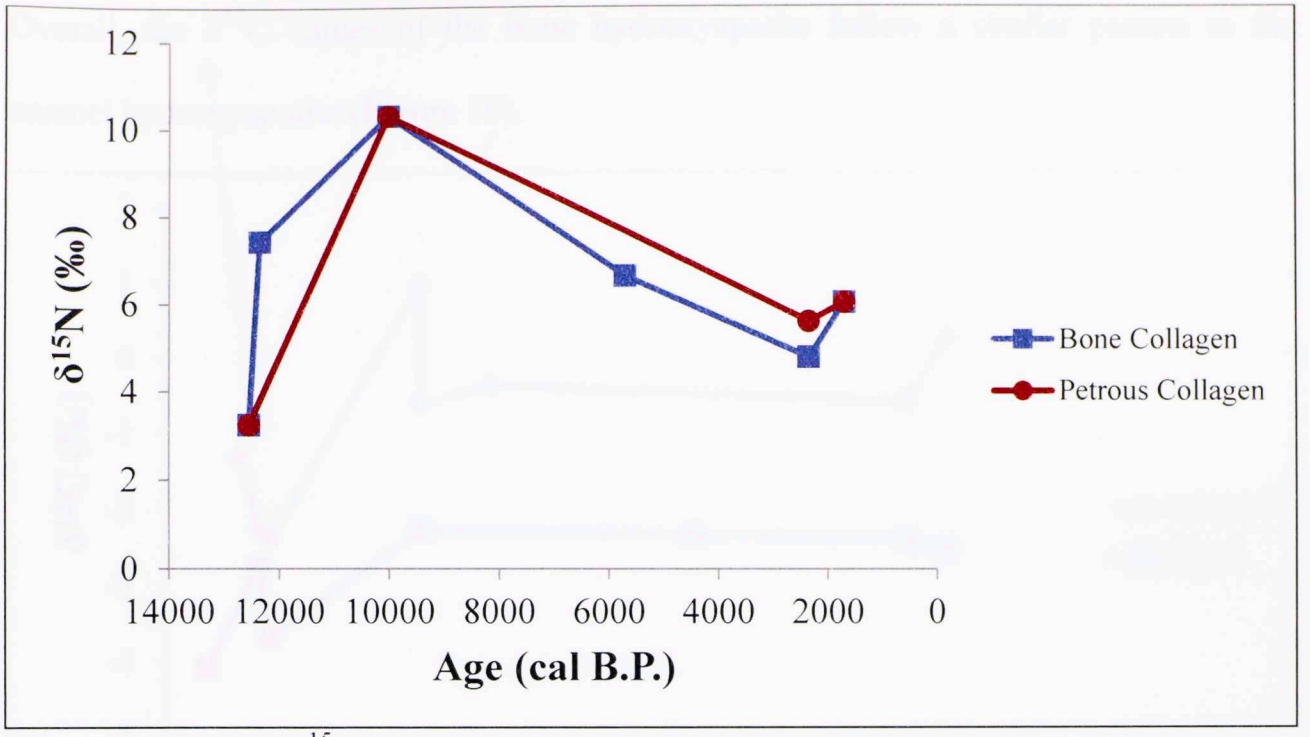


**Figure 9**. Average $\delta^{15}N$ values per site of collagen from all bone samples (blue squares) and just petrous samples (red circles), excluding values determined unreliable by amino acid analysis.

*Stable Isotope Analysis of Enamel and Bone Hydroxyapatite*

The $\delta^{13}C$ value from the enamel hydroxyapatite from the Jake Bluff site (12,850 cal B.P.) is -1.31. The average $\delta^{13}C$ values become depleted until 12,350 cal B.P., then become enriched in $^{13}C$ to a maximum average of 0.96 ± 0.07 at the Ravenscroft site (10,000 cal B.P.). The same-age site, Bull Creek (10,000 cal B.P.), is slightly more depleted, with an average $\delta^{13}C$ value of -0.63 ± 0.00. The $\delta^{13}C$ values remain until 2,350 cal B.P., then become enriched in $^{13}C$ at 1,700 cal B.P., then depleted in $^{13}C$ at the present (Figure 10).

The average of the $\delta^{13}C$ values from the bone hydroxyapatite stay slightly more depleted until the present, where they are slightly more enriched than the enamel hydroxyapatite $\delta^{13}C$ values. There is an additional $\delta^{13}C$ value for the Military Trail site (13,350 cal B.P.) which averages -4.10 ± 0.28, lighter than the Jake Bluff average.

Overall, the $\delta^{13}C$ values of the bone hydroxyapatite follow a similar pattern to the enamel hydroxyapatite (Figure 10).

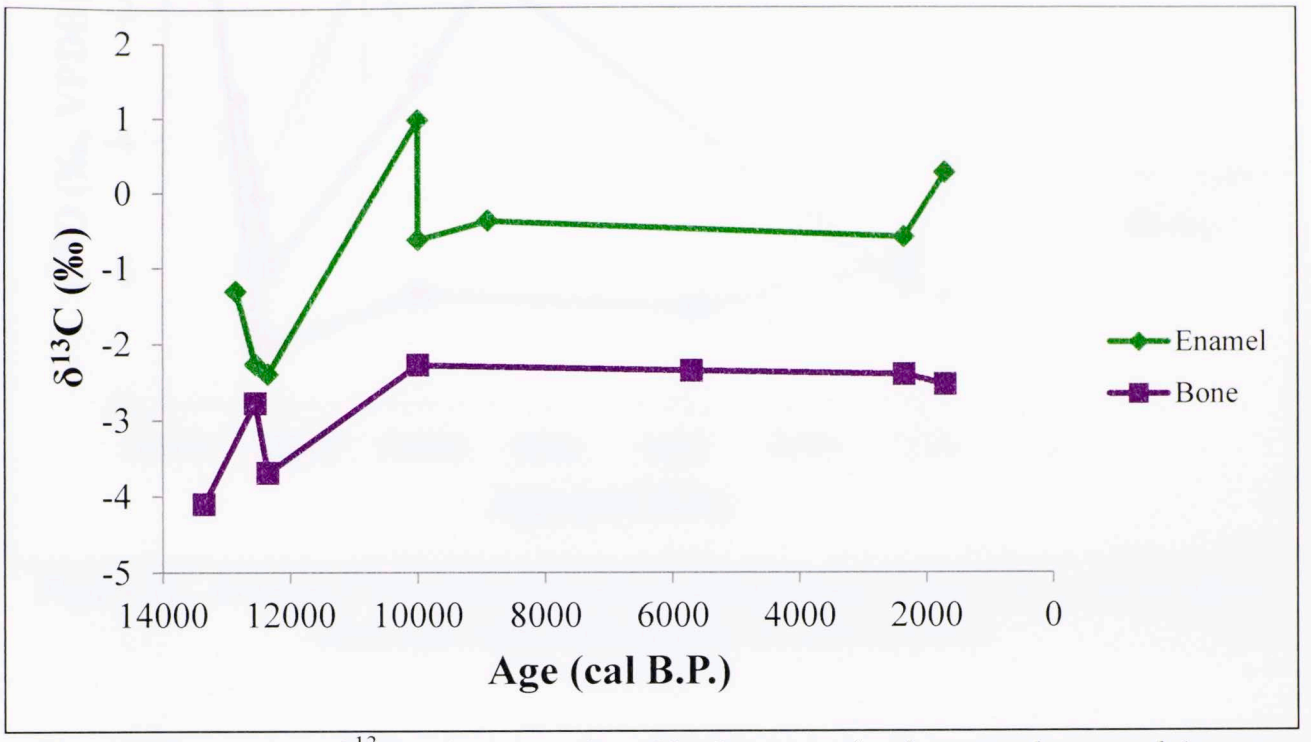


**Figure 10**. Average $\delta^{13}C$ values per site of hydroxyapatite from tooth enamel (green diamonds) and bone samples (purple squares).

The $\delta^{18}O$ value of the enamel hydroxyapatite from the Jake Bluff site is -3.39. The average $\delta^{18}O$ values become lighter until 12,350 cal B.P. then gradually become heavier to a maximum average of -1.48 ± 0.01 at 8,900 cal B.P. The values become depleted in $^{18}O$ until 1,700 cal B.P. and then become enriched in $^{18}O$ at the present (Figure 11).

The average $\delta^{18}O$ values of the bone hydroxyapatite are slightly more depleted than the enamel hydroxyapatite except at 2,350 cal B.P. and 1,700 cal B.P. An additional $\delta^{18}O$ value for the Military Trail site (13,350 cal B.P.) of -0.44 ± 0.65 is the heaviest average value (Figure 11).

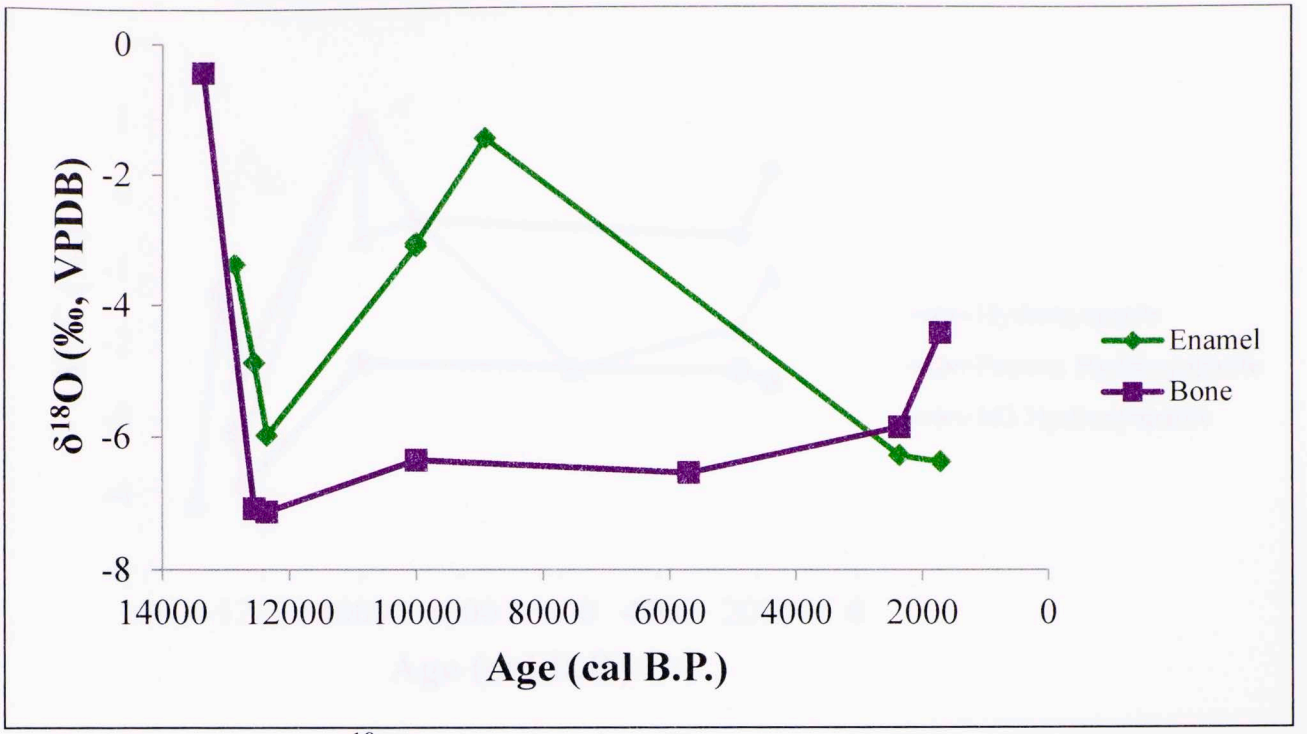


**Figure 11**. Average $\delta^{18}O$ values per site of hydroxyapatite from tooth enamel (green diamonds) and bone samples (purple squares).

The combined $\delta^{13}C$ average values of both the enamel and bone hydroxyapatite are shown in Figure 12. The average $\delta^{13}C$ values of just petrous hydroxyapatite are also plotted. These values follow a similar pattern but are slightly more depleted than the total averages and averages of the third molars. The third molar average $\delta^{13}C$ values also show a similar pattern but are more enriched than both the total and petrous averages, with the exception of the present, where the average third molar value is slightly more depleted in $^{18}O$ than the average of the total hydroxyapatite. The use of the third molar is due to the availability of this particular tooth at most sites and because its isotopic values are representative of the animal's post-weaning diet (Larson et al. 2001 and references therein).

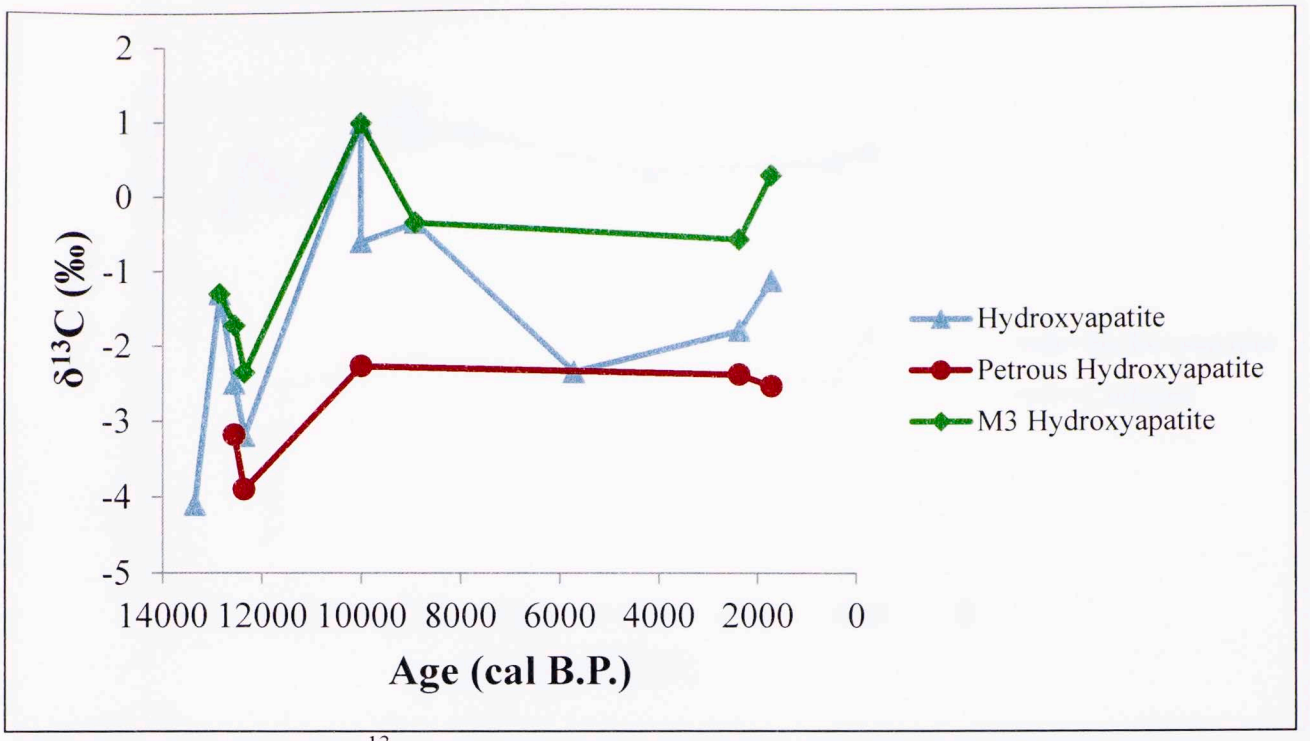


**Figure 12**. Average $\delta^{13}C$ values per site of hydroxyapatite from all bone and tooth samples (aqua triangles), just petrous samples (red circles), and third molars (green diamonds).

Figure 13 shows the average $\delta^{13}C$ values of the hydroxyapatite and collagen from every well-preserved bone and tooth sample. Shifts between the hydroxyapatite and collagen values can be seen. The hydroxyapatite values are approximately 8 to 18 per mil more enriched in $^{13}C$ than the collagen values. As will be discussed below, this variability may reflect differences in the respective carbon sources and/or rates of metabolic turnover for carbon associated with hydroxyapatite vs. collagen in the organism, e.g., Jahren et al. (1998).

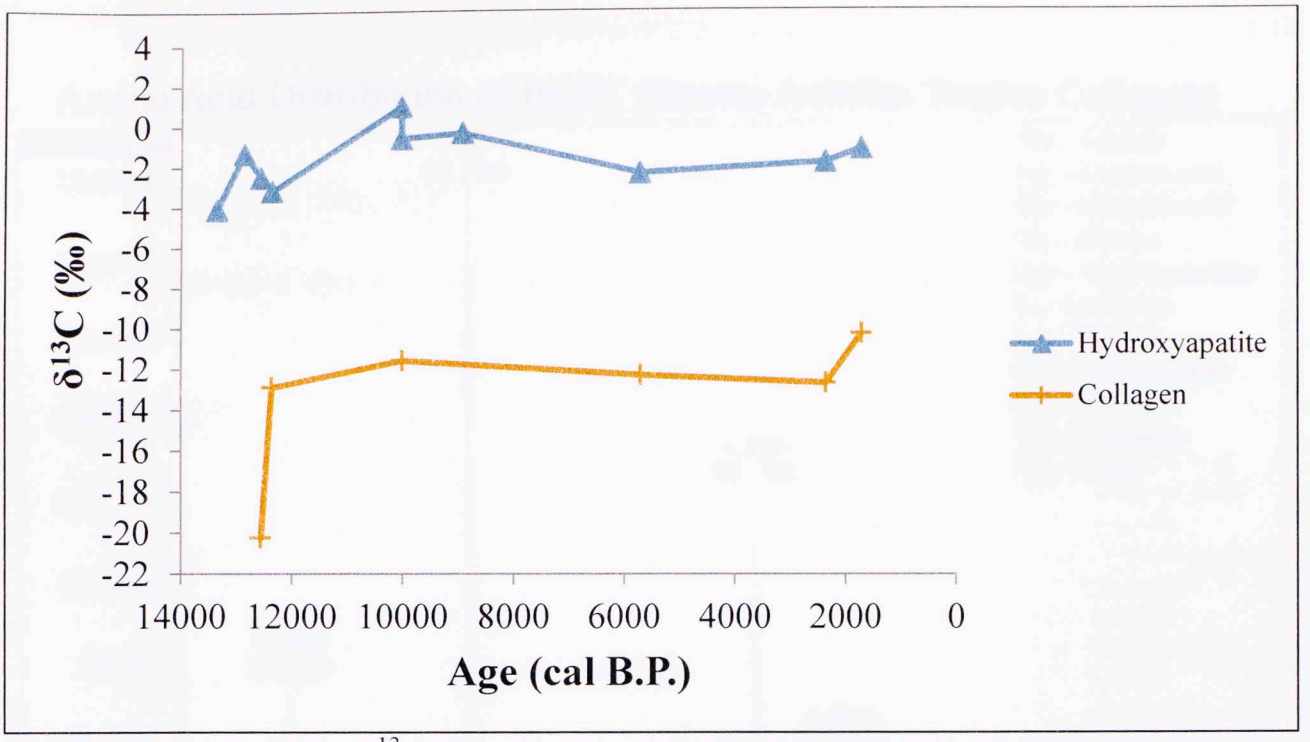


**Figure 13**. Average $\delta^{13}C$ values per site of all bone and tooth samples, excluding unreliable values, separated into hydroxyapatite (aqua triangles) and collagen (orange crosses).

*GC/MS of Amino Acids*

The relative amino acid abundances from the chromatograms of both modern and fossil bison samples were compared to the chromatogram of the control sample (bovine achilles tendon collagen, BATC) in order to determine collagen preservation and whether isotopic values from the collagen could be reliable. Figure 14 shows the presence of 11 protein amino acids characteristic of BATC. They are: alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), hydroxyproline (Hyp), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), proline (Pro), threonine (Thr), and valine (Val). The four most abundant amino acids are Ala, Gly, Hyp, and Pro.

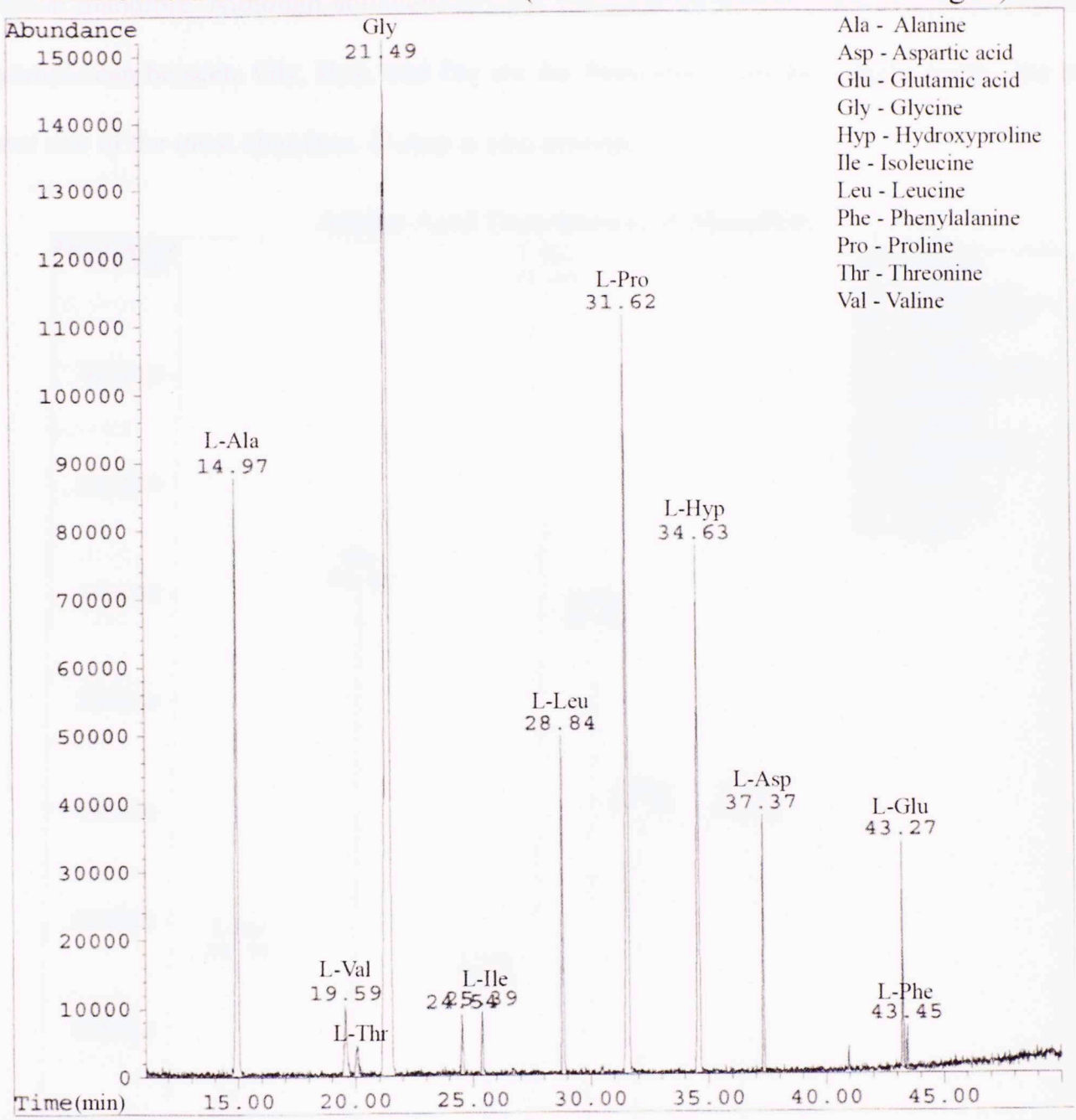


**Figure 14**. Chromatogram of amino acid abundances in modern bovine achilles tendon collagen (Alfa Aesar, Ward Hill, MA).

Figure 15 shows the amino acid distribution for the collagen of the modern bison mandible. Although abundances vary, the same 11 amino acids as in the control sample can be seen. Gly, Hyp, and Pro are the three most abundant amino acids; Ala is not one of the most abundant. D-Asp is also present.

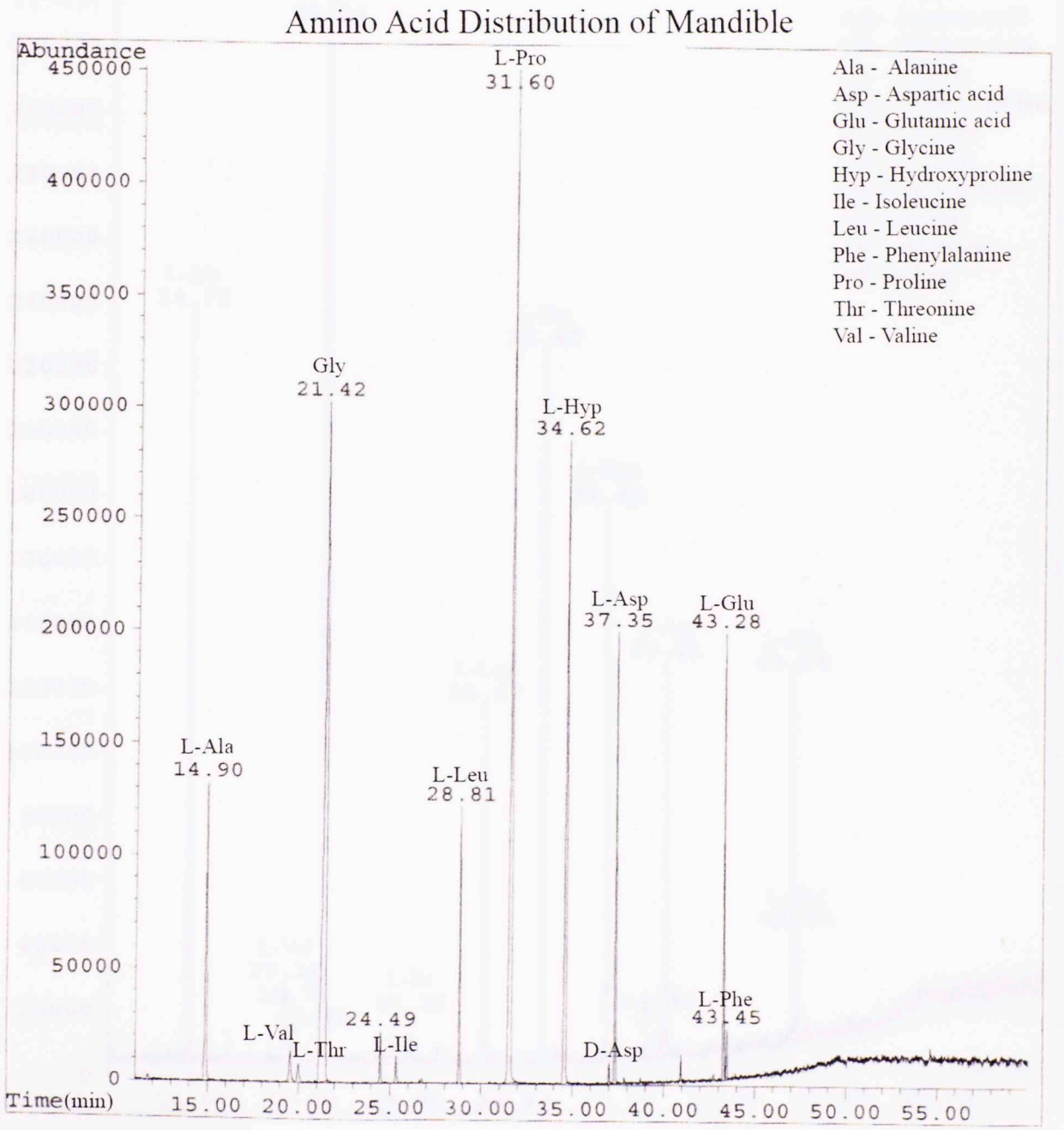


**Figure 15**. Chromatogram of amino acid abundances in the collagen isolated from the modern bison mandible.

Figure 16 shows the amino acid distribution for the collagen of the petrous bone from the Certain site. Once again, the same 11 amino acids are present with the most abundant being Ala, Gly, Hyp, and Pro.

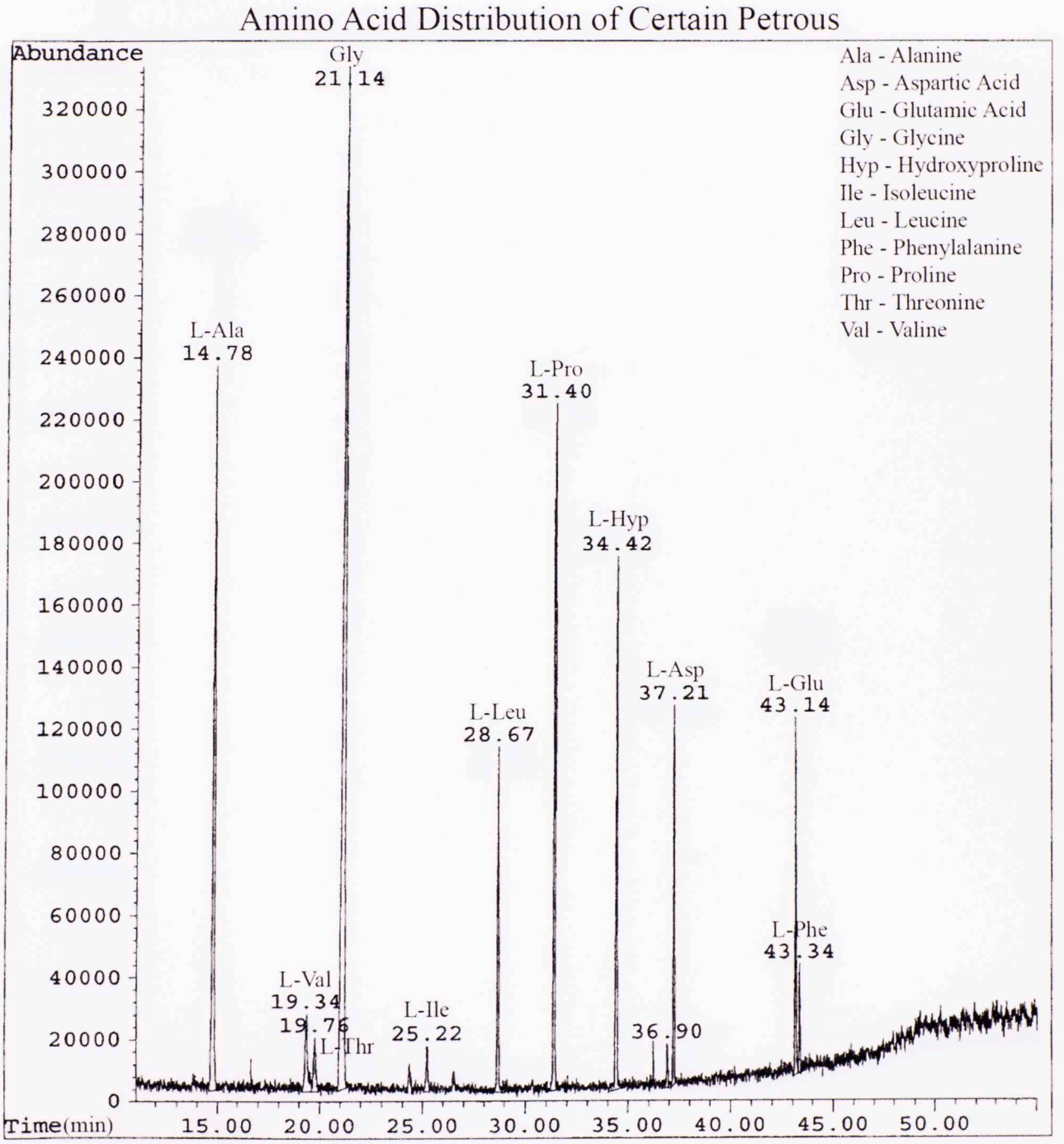


**Figure 16**. Chromatogram of amino acid abundances in the collagen isolated from the petrous bone from the Certain site.

Figure 17 shows the amino acid distribution for the collagen of the petrous bone from the Harrel site. The same 11 amino acids are again present with the most abundant being Ala, Gly, Hyp, and Pro.

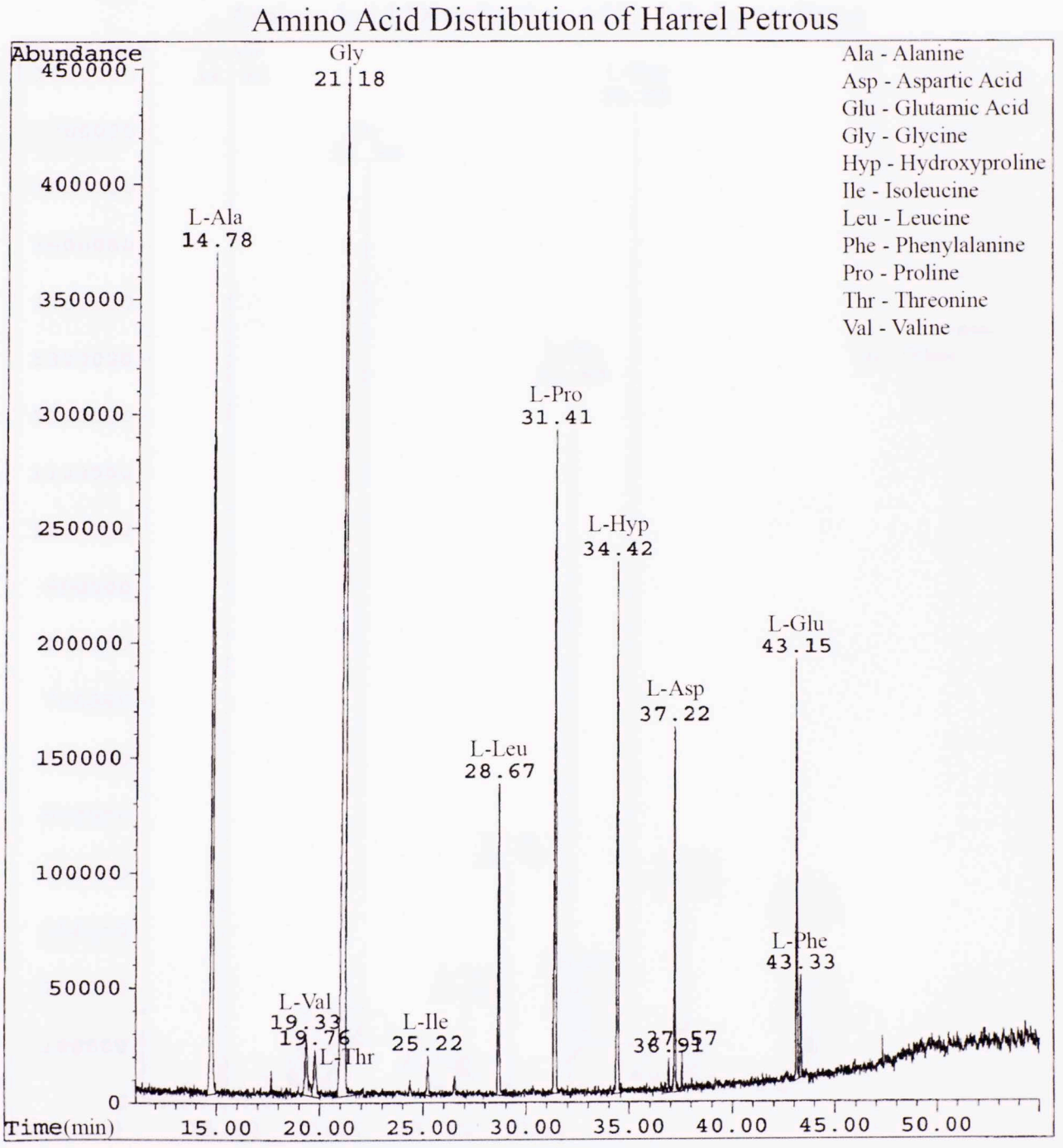


**Figure 17**. Chromatogram of amino acid abundances in the collagen isolated from the petrous bone from the Harrel site.

Figure 18 shows the amino acid distribution for the collagen of the long bone from the Kubik site. There are 12 amino acids, the addition being Ser. The same four are most abundant: Ala, Gly, Hyp, and Pro.

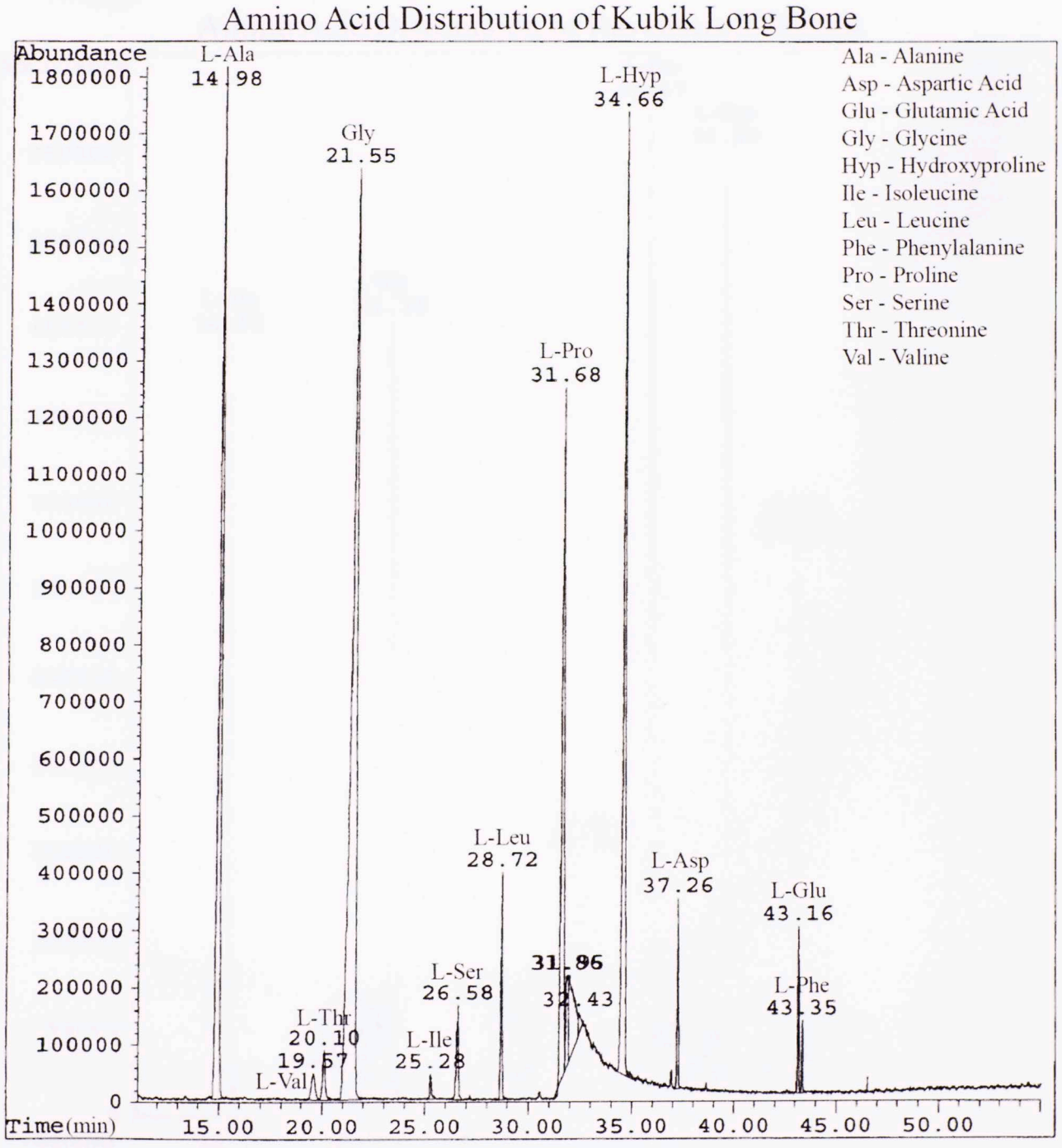


**Figure 18**. Chromatogram of amino acid abundances in the collagen isolated from the long bone from the Kubik site.

Figure 19 shows the amino acid distribution within the collagen of the petrous bone from the Ravenscroft site. The same 11 amino acids are present, but Glu is also among the most abundant. D-Asp is also present.

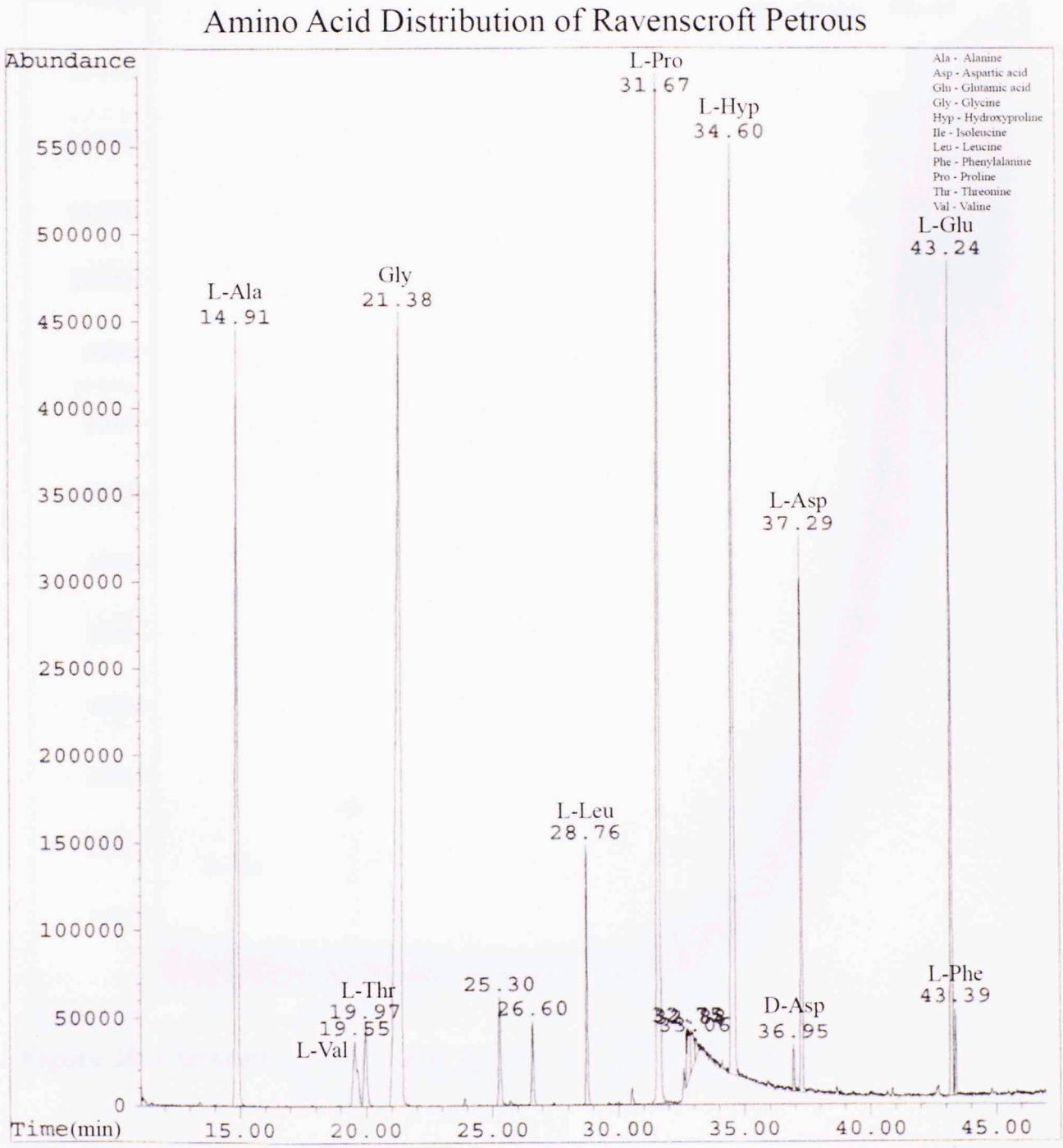


**Figure 19**. Chromatogram of amino acid abundances in the collagen isolated from the petrous bone from the Ravenscroft site.

Figure 20 shows the amino acid distribution for the collagen of the second molar from the Badger Hole site. Only two amino acids are present: Ala and Gly.

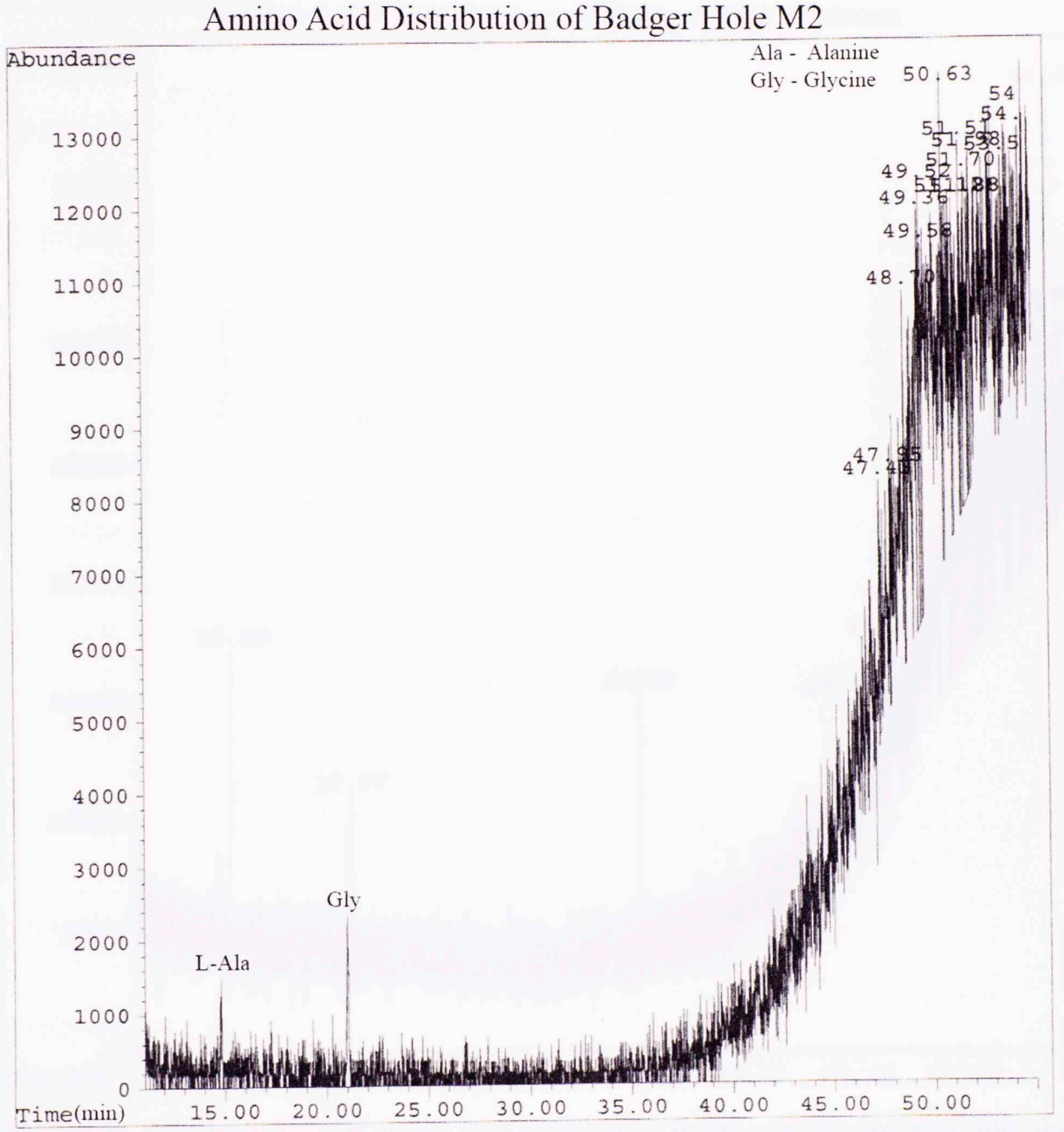


**Figure 20**. Chromatogram of amino acid abundances in the collagen isolated from the second molar from the Badger Hole site.

Figure 21 shows the amino acid distribution for the collagen of the petrous bone from the Badger Hole site. No amino acids are present.

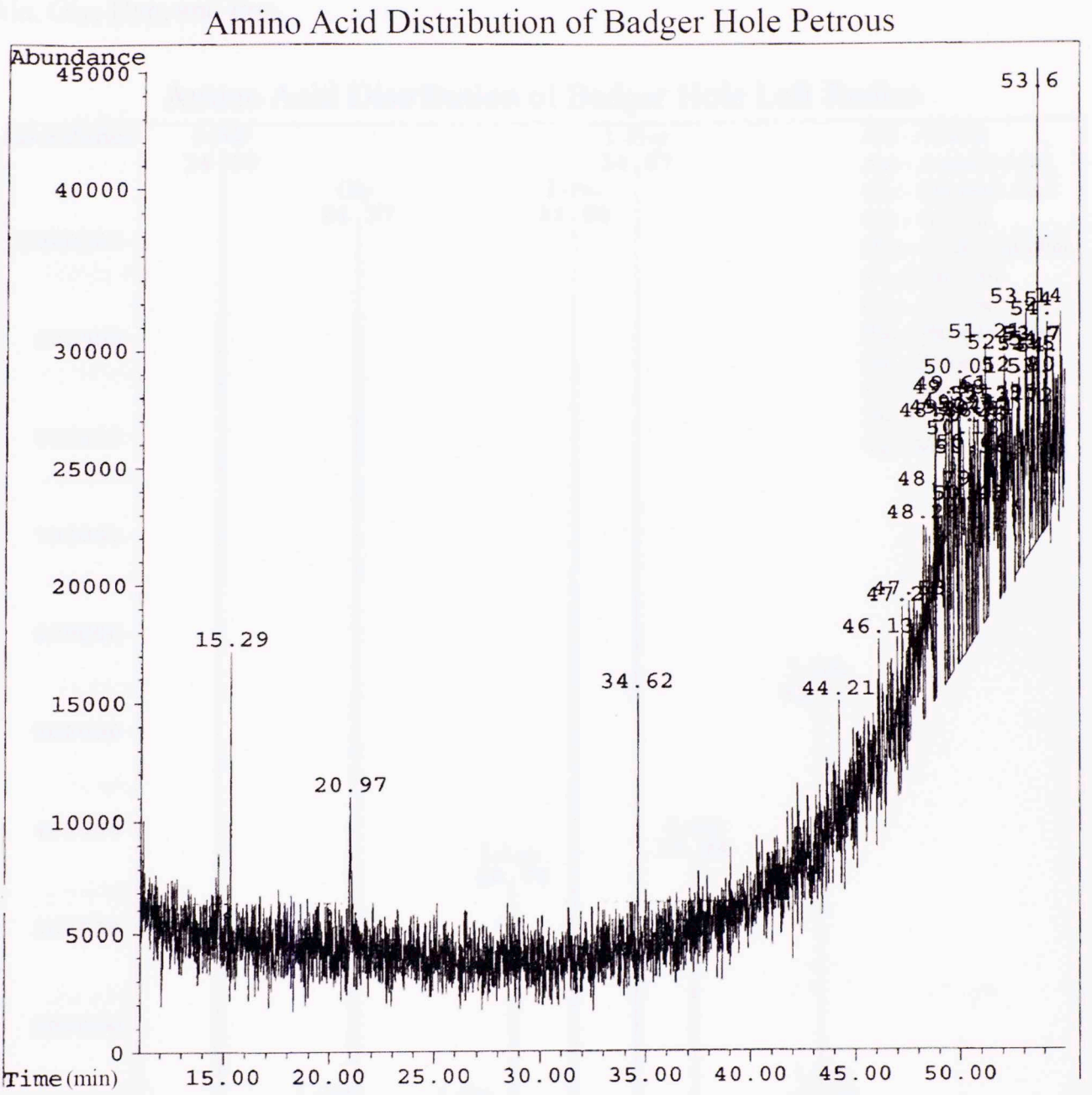


**Figure 21**. Chromatogram of amino acid abundances in the collagen isolated from the petrous bone from the Badger Hole site.

Figure 22 shows the amino acid distribution for the collagen of the left radius from the Badger Hole site. There are 12 amino acids present and the most abundant are Ala, Gly, Hyp, and Pro.

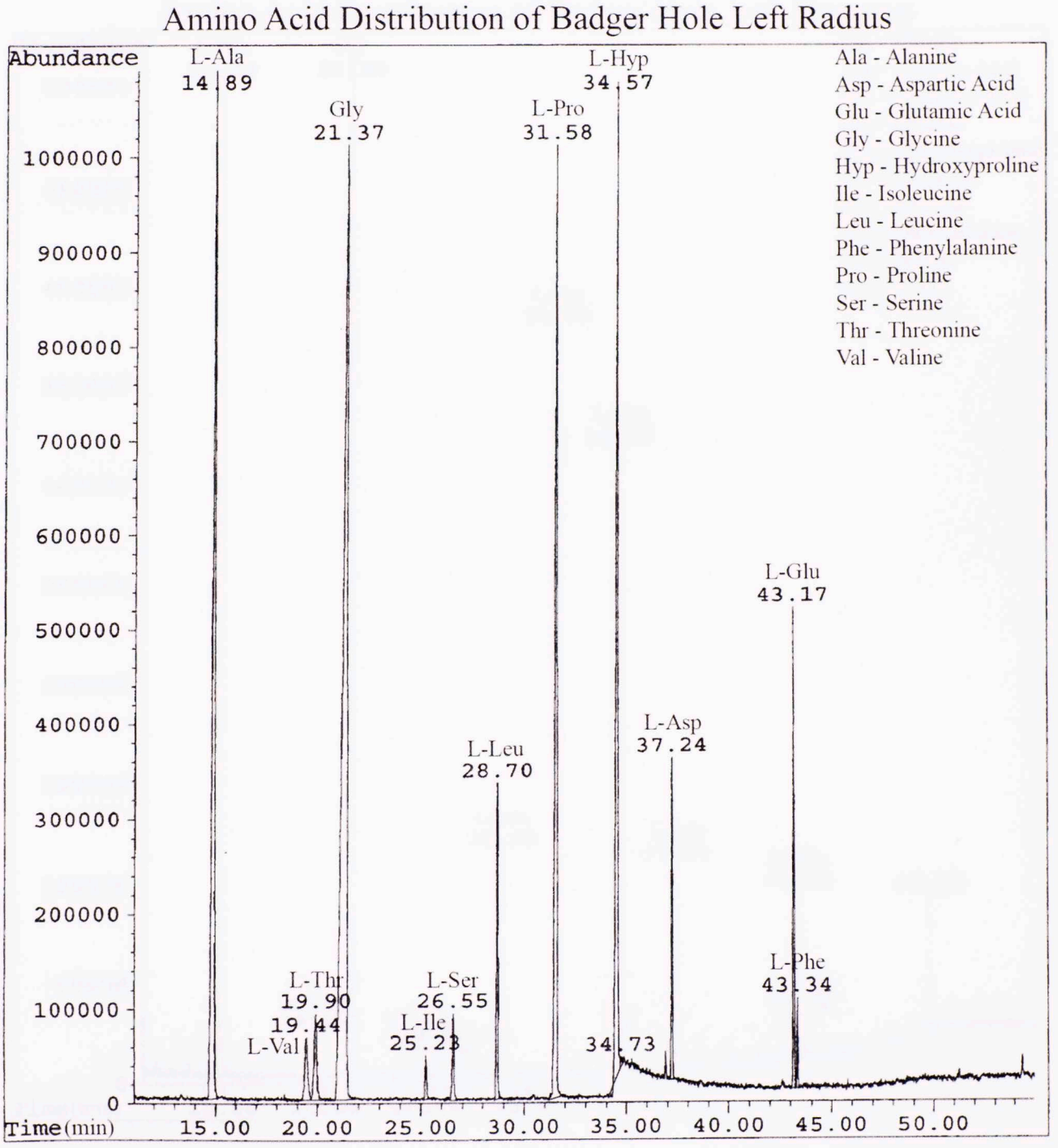


**Figure 22**. Chromatogram of amino acid abundances in the collagen isolated from the left radius from the Badger Hole site.

Figure 23 shows the amino acid distribution for the collagen of the left humerus from the Badger Hole site. There are 10 amino acids present; Thr is absent. The most abundant amino acids are Ala, Gly, Hyp, and Pro.

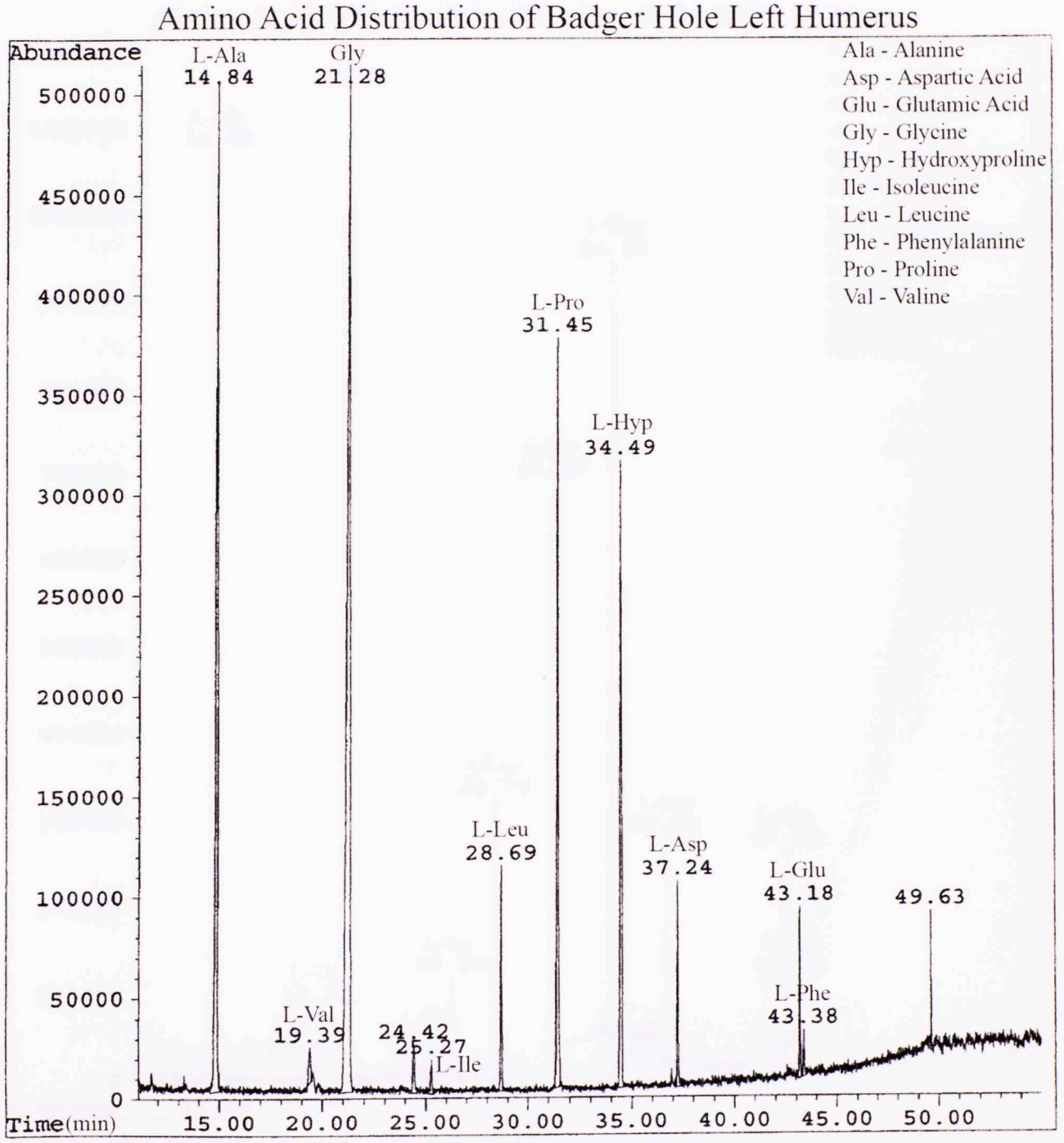


**Figure 23**. Chromatogram of amino acid abundances in the collagen isolated from the left humerus from the Badger Hole site.

Figure 24 shows the amino acid distribution for the collagen of the right humerus from the Badger Hole site. There are 12 amino acids present. Ala, Gly, Hyp, and Pro are the most abundant.

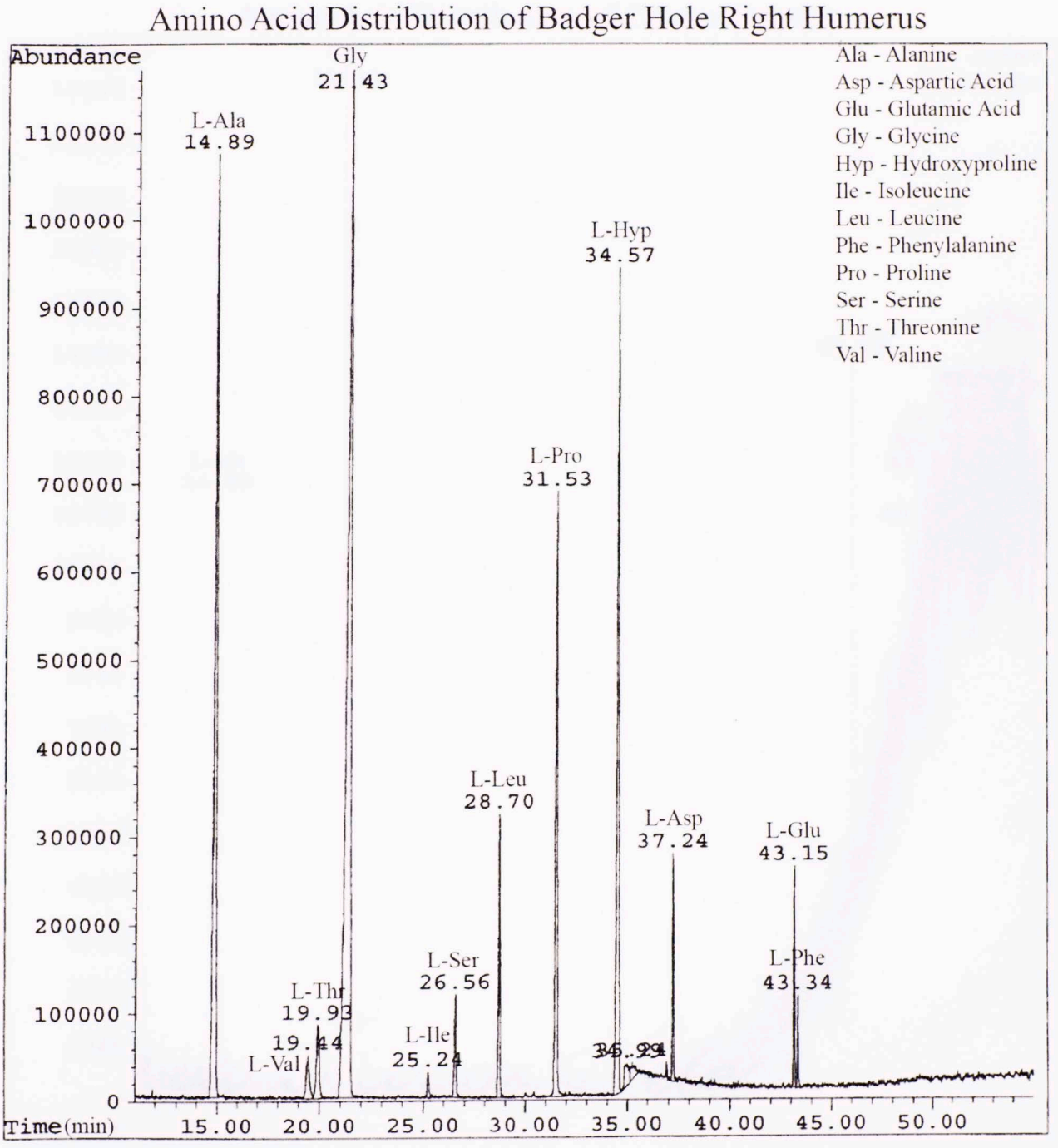


**Figure 24**. Chromatogram of amino acid abundances in the collagen isolated from the right humerus from the Badger Hole site.

Figure 25 shows the amino acid distribution for the collagen of one of the petrous bones from the Cooper site. There are only two amino acids present: Ala and Gly.

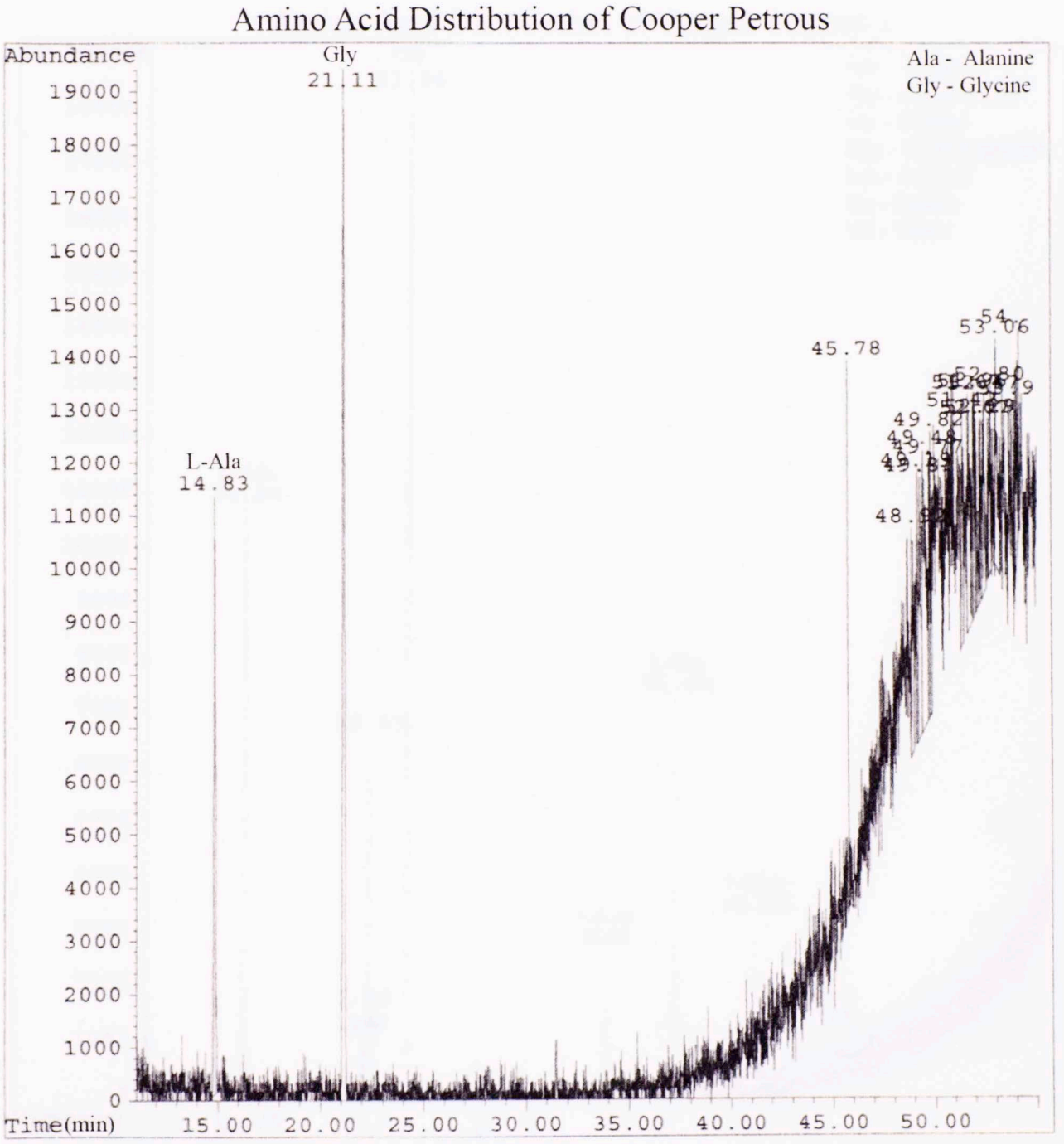


**Figure 25**. Chromatogram of amino acid abundances in the collagen isolated from the petrous bone from the Cooper site.

Figure 26 shows the amino acid distribution for the collagen of a second petrous bone from the Cooper site. There are seven amino acids present. Ala, Gly, Hyp, and Pro are the most abundant.

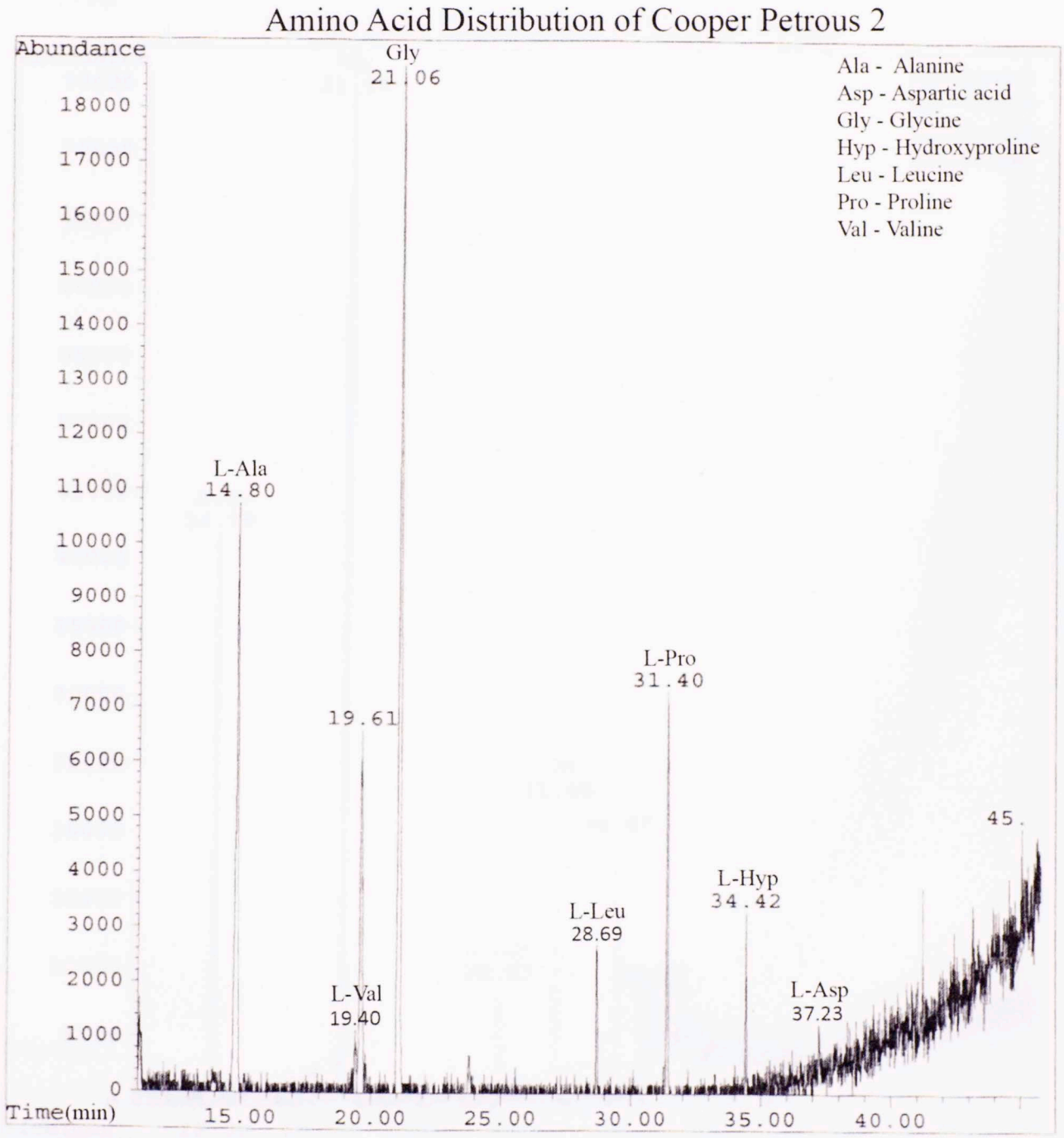


**Figure 26**. Chromatogram of amino acid abundances in the collagen isolated from the second petrous bone from the Cooper site.

Figure 27 shows the amino acid distribution for the collagen of the femur from the Cooper site. There are only four amino acids present: Ala, Gly, Leu, and Pro; Hyp is absent.

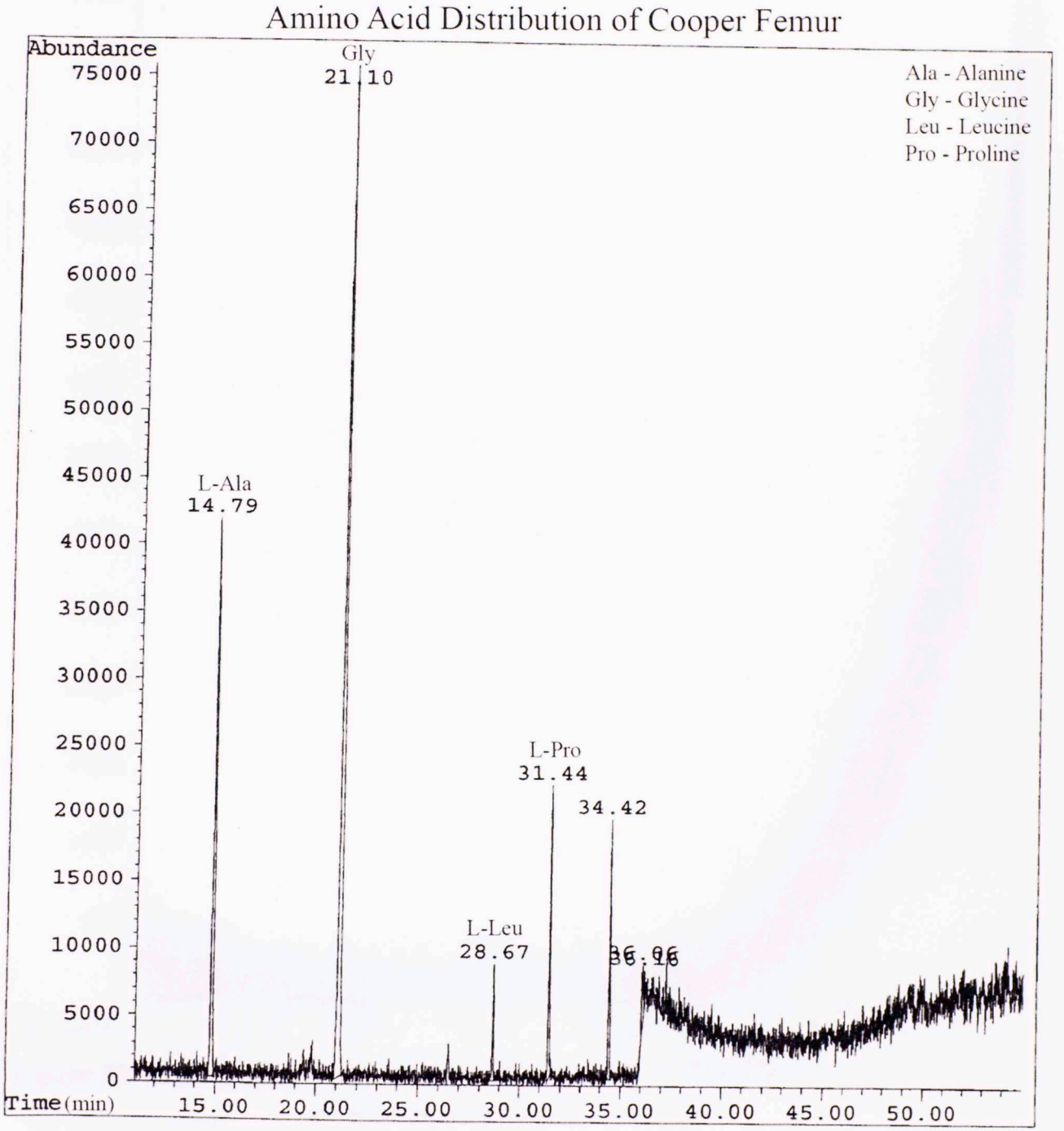


**Figure 27**. Chromatogram of amino acid abundances in the collagen isolated from the femur from the Cooper site.

Figure 28 shows the amino acid distribution for the collagen of the third molar from the Jake Bluff site. No amino acids are present.

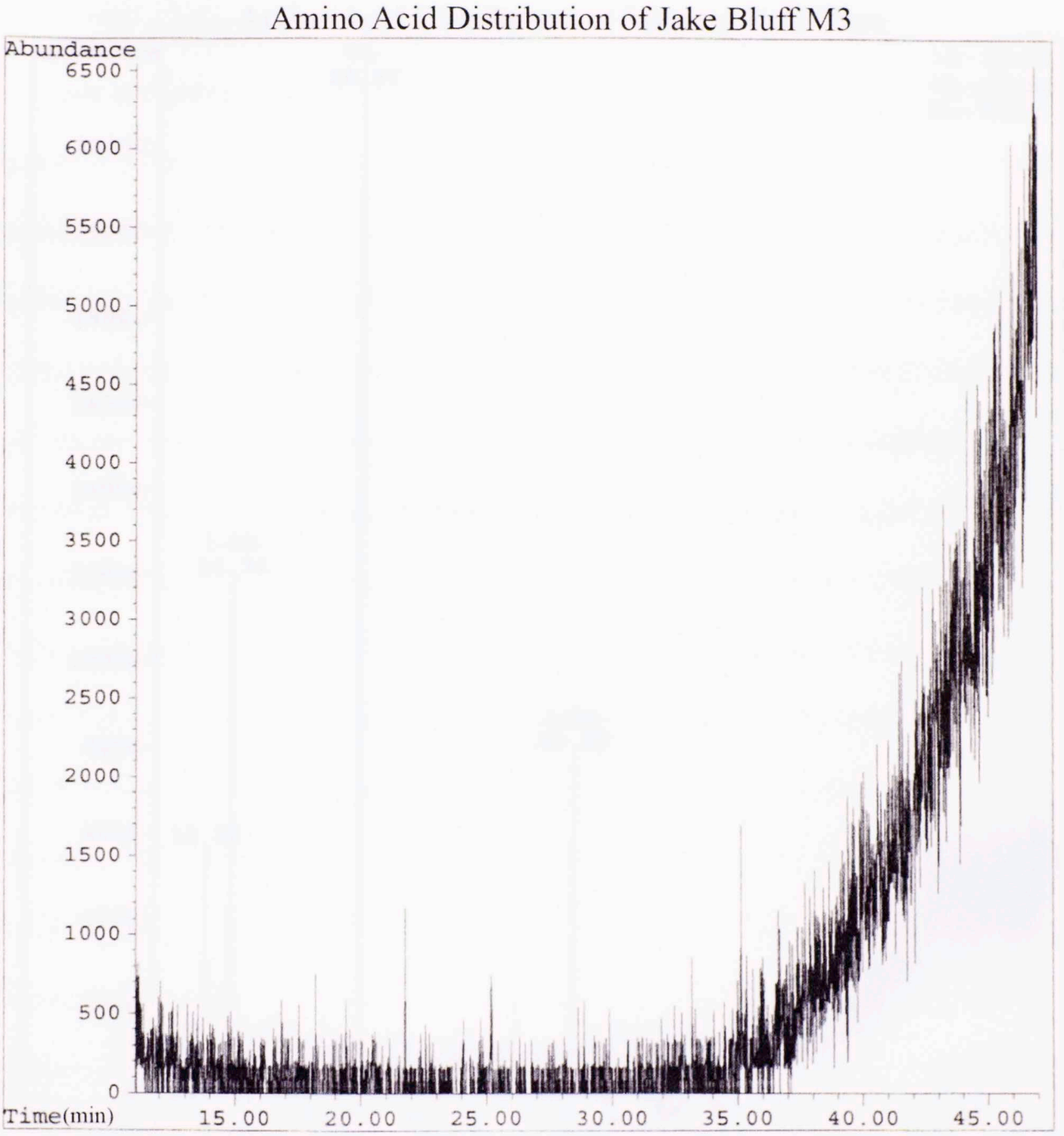


**Figure 28**. Chromatogram of amino acid abundances in the collagen isolated from the third molar from the Jake Bluff site.

Figure 29 shows the amino acid distribution for the collagen of the tibia from the Military Trail site. Only three amino acids are present; Hyp is absent.

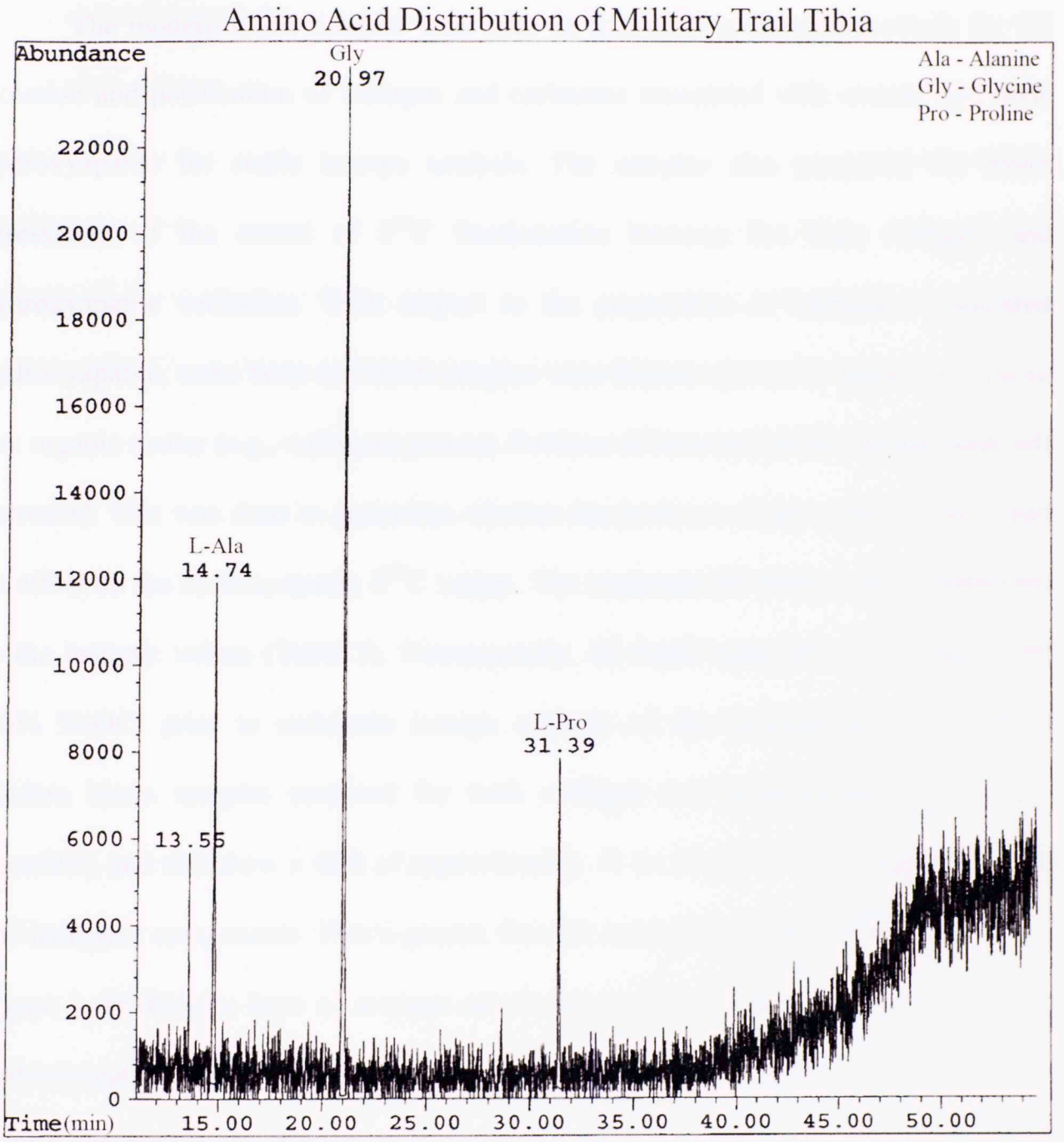


**Figure 29**. Chromatogram of amino acid abundances in the collagen isolated from the tibia from the Military Trail site.

# Discussion

*Modern Samples*

The modern bison samples were used in this study to develop methods for the isolation and purification of collagen and carbonate associated with enamel and bone hydroxyapatite for stable isotope analysis. The samples also permitted the direct assessment of the extent of $\delta^{13}C$ fractionation between the bone collagen and hydroxyapatite carbonate. With respect to the preparation of carbonate associated hydroxyapatite, some bone and tooth samples were treated with 2.5% NaOCl to remove any organic matter (e.g., collagen) present. Portions of bone and tooth samples were left untreated. This was done to determine whether the presence of any organic matter had an effect on the hydroxyapatite $\delta^{13}C$ values. The treatment did make a slight difference in the isotopic values (Table 3). Consequently, all fossil materials were treated with 2.5% NaOCl prior to carbonate isotope analysis of the hydroxyapatite. The three modern bison samples analyzed for both collagen and hydroxyapatite $\delta^{13}C$ (M2, mandible, and rib) show a shift of approximately 11 to 14 per mil between the organic and inorganic components. This is greater than the average shift shown for herbivores in Figure 1. Herbivores have an average enrichment in $\delta^{13}C$ values of 7 to 9 per mil for hydroxyapatite relative to collagen (Koch et al. 1997 and references therein). The results observed in the present study for bison could be indicative of the difference in metabolic turnover rates between collagen and hydroxyapatite. Collagen values are representative of a single growth season signal, whereas hydroxyapatite values are representative of a long-term growth signal (Jahren et al. 1998). The absolute $\delta^{13}C$ values for collagen and hydroxyapatite of $C_3$ vs. $C_4$ feeders shown in Figure 1 are based

on the pre-industrial $\delta^{13}C$ value of atmospheric $CO_2$ (-6.5 ‰) and thus cannot be directly applied to the $\delta^{13}C$ values of the modern samples in this study, which reflect the $\delta^{13}C$ value of present-day atmospheric $CO_2$ (~-8.5 ‰). However, the depleted modern $\delta^{13}C$ values show a mostly $C_3$ diet with a $C_4$ influence, indicating a mixed diet. The modern $\delta^{13}C$ values for each bone and tooth sample are very similar due to the fact they are from the same organism. The second molar sample (M2) has slightly more enriched collagen $\delta^{13}C$ values than the mandible and rib. This is possibly due to the fact that the second molar forms during weaning (Larson et al. 2001).

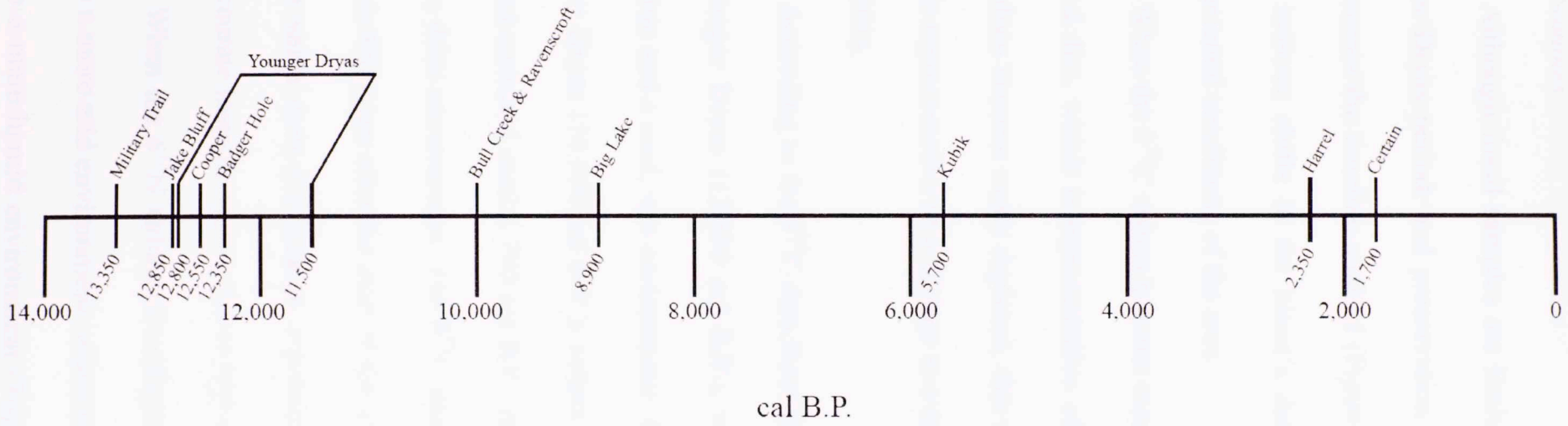


**Figure 30**. Timeline with fossil kill sites and their ages.

*Fossil Samples*

Although fossil samples are limited, especially those from sites older than the Younger Dryas period, and preservation of samples varies, a distinct shift in isotopic values across the timeline studied (Figure 30) can be seen (Figures 8 to 13). The $\delta^{13}C$ values indicate shifts in the bison's diet, which may ultimately indicate a shift in environmental conditions of the area.

When the $\delta^{13}C$ values become more enriched, this may indicate a shift to a more $C_4$-based diet, which is representative of a change to a warmer, drier climate. When $\delta^{13}C$ values become more depleted, this may indicate a shift to a more $C_3$-based diet, which is representative of a change to a cooler, wetter climate (Hare et al. 1991; Larson et al. 2001).

According to the $\delta^{13}C$ data from the petrous collagen, 250 years after the start of the Younger Dryas (12,550 cal B.P.), values were depleted, indicating primarily $C_3$ vegetation and a cool, wet environment. Approximately 1,500 years after the end of the Younger Dryas (10,000 cal B.P.), values became enriched by more than 8 per mil and remained enriched until 1,700 cal B.P., indicating a shift to a more $C_4$-based diet and a warmer, drier environment. The $\delta^{13}C$ data from the bone collagen show an enrichment in values 450 years after the start of the Younger Dryas (12,350 cal B.P.). However, this average value does not include a petrous sample, which was determined to give the most accurate isotopic values due to best collagen preservation (Figure 8).

When the $\delta^{15}N$ values of collagen become more enriched, this may indicate a shift to a more arid environment, whereas a depletion in the $\delta^{15}N$ values may indicate a shift to a more humid environment (Topalov et al. 2012 and references therein). The

$\delta^{15}N$ values from the bone collagen (including petrous samples) (Figure 9) follow the same enrichment and depletion pattern as the $\delta^{13}C$ values from the bone collagen (Figure 8), i.e., at times when the $\delta^{13}C$ values showed greater $C_3$ or $C_4$ vegetation abundances, the climate was wetter or drier, respectively (Figure 9).

According to the $\delta^{13}C$ data from the combined enamel and bone hydroxyapatite, 550 years before the start of the Younger Dryas (13,350 cal B.P.), values were depleted indicating a cool, wet climate dominated by $C_3$ vegetation. Approximately 50 years before the start of the Younger Dryas (12,850 cal B.P.), $\delta^{13}C$ values became more enriched, indicating a shift to warmer, drier conditions and an increase in $C_4$ vegetation. Throughout the Younger Dryas, $\delta^{13}C$ values become more depleted indicating a gradual shift to more $C_3$ vegetation and cooler, wetter environmental conditions (Figure 12). From 12,350 cal B.P. until present, the combined enamel and bone hydroxyapatite $\delta^{13}C$ values match the same shifts in climate and vegetation as the collagen $\delta^{13}C$ values (Figure 8). The greater 8 to 18 per mil shift between the collagen and hydroxyapatite $\delta^{13}C$ values in this study may indicate the difference in turnover rates for carbon in these two materials and/or the seasonality of the collagen values vs. the long-term hydroxyapatite values (e.g., Jahren et al. 1998).

When $\delta^{18}O$ values of meteoric water become more enriched, this is indicative of an increase in temperature, whereas a depletion in $\delta^{18}O$ values indicates a decrease in temperature. This is because precipitation generally becomes more depleted in $\delta^{18}O$ with increasing latitude and decreasing temperature. Carbonates precipitating in equilibrium with water will reflect this trend (Wright and Schwarcz 1998; Sharp 2007 and references therein). The $\delta^{18}O$ values from the combined enamel and bone carbonate

associated with the hydroxyapatite do not follow a similar pattern of enrichment and depletion as the $\delta^{13}C$ values for the combined enamel and bone hydroxyapatite. The $\delta^{18}O$ values of hydroxyapatite are not as reliable as $\delta^{13}C$ values, especially enamel hydroxyapatite, in recording original isotopic signatures because $\delta^{18}O$ values are more susceptible to diagenesis (Wang and Cerling 1994). Also, they may likely reflect the isotopic variability associated with local water sources (Kohn and McKay 2012). The slight shifts in the $\delta^{13}C$ and $\delta^{18}O$ values between the enamel and bone hydroxyapatite could be due to the fact that all of the fossil samples are from different animals.

*Collagen Preservation*

The collagen samples that had the more abundant protein amino acids, relative to the control sample (BATC) (Figure 14), especially Hyp, were considered more well-preserved (Figures 15 to 19, 22 to 24, and 26) and were used in the calculations for the averages plotted in the graphs (Figures 8 to 13). The well-preserved samples all contained the same 11 amino acids as BATC with either the addition of Ser (Figures 18, 22, and 24) or the absence of Thr (Figure 23). Threonine and Ser are relatively unstable amino acids. Their concentrations will vary as a function of the type of local preservation conditions (e.g., Macko et al. 1999). Also, the protein amino acids racemize at varying rates (Griffin et al. 2010 and references therein). Aspartic acid racemizes the fastest; thus, even in modern bone and teeth and/or relatively young fossil bone and teeth it is not uncommon to see a small amount of D-Asp present in the chromatograms (e.g., Figures 15 and 19). The abundances of the amino acids varied, but Ala, Gly, Hyp, and Pro were always the most abundant, just as expected according to

Table 1 and BATC. The amino acid Hyp is primarily found in collagen and is useful for determining the preservation of collagen (Ambrose and Krigbaum 2003).

The second molar from the Badger Hole site, the petrous bone and femur from the Cooper site, and the tibia from the Military Trail site all contained a few amino acids, but Hyp was absent (Figures 20, 25, 27, and 29); thus, the collagen in these samples was considered not well-preserved. The petrous bone from the Badger Hole site and the third molar from Jake Bluff showed no presence of any amino acids and were also considered to have poor collagen preservation (Figures 21 and 28). The isotopic values obtained from the collagen of these samples were not used in calculating the averages plotted in the graphs (Figures 8, 9, and 13).

Poor preservation of the collagen from these samples could be due to several factors. Collagen loss is due to time, temperature, and biological factors. The older a bone is, the more degraded it is, and these samples are pre-Holocene in age. Porosity plays a role – bones are more porous than teeth and are more susceptible to diagenetic alteration. Hotter climates increase collagen loss. Microbial attack also increases collagen loss. Hydrology affects microbial attack. Variations in moisture may increase microbial attack and ultimately increase collagen loss (e.g., Hedges 2002). Because these samples were found in arroyos, it can be assumed that moisture variation was a factor contributing to the likelihood of diagenesis.

*Comparison to Previous Bison Studies*

Numerous investigators have attempted to reconstruct paleodiet and paleoclimate conditions based on the stable isotope compositions of organic and inorganic constituents of bison bone and teeth (e.g., Hare et al. 1991; Tieszen 1994;

Connin et al. 1998; Koch 1998; Koch et al. 1998; Jahren et al. 1998; Gadbury et al. 2000; Larson et al. 2001; Passey and Cerling 2002; Koch et al. 2004; Hoppe et al. 2006; Bernard et al. 2009; Clementz et al. 2009; Widga et al. 2010; Kohn and McKay 2012). However, these studies did not examine bison samples from Oklahoma during the Younger Dryas, a period of abrupt climate cooling that occurred approximately 12,800 cal B.P. and lasted 1,300 ± 70 years, or across the timeline of the present study.

Of the prior studies, Jahren et al. (1998) is the only study to examine the isotopes in both the organic and inorganic components of bison bones and teeth near the timeline of this present study. Eleven bison rib bones were collected from the Hudson-Meng Bonebed, which is located in Sioux County in northwestern Nebraska. The site is dated to be early Holocene, approximately 9,500 $^{14}$C yr B.P. or 10,700 cal B.P. The $\delta^{13}$C values of the bone collagen from these samples ranged from -15.58 to -21.88 ‰, with a mean of -18.02 ‰. Three samples did not contain sufficient collagen for isotopic measurements. Preservation of samples was determined using microscopy. The $\delta^{15}$N values of the bone collagen were not measured. The $\delta^{13}$C values of the bone carbonate within the hydroxyapatite ranged from -9.24 to -6.63 ‰, with a mean of -7.5 ‰. The $\delta^{18}$O values (in VSMOW) ranged from 21.70 to 24.00 ‰ (-8.89 to -6.65 ‰ VPDB), with a mean of 22.9 ‰ (-7.72 ‰ VPDB). Twenty-eight bison teeth (including 1$^{st}$, 2$^{nd}$, and 3$^{rd}$ molars) were also collected and yielded $\delta^{13}$C and $\delta^{18}$O (in VSMOW) mean values of -5.96 ‰ and 21.93 ‰ (-8.66 VPDB), respectively, from the enamel carbonate within the hydroxyapatite. The author stated that these values indicated an abundance of $C_4$ vegetation, suggesting a warmer, drier environment at this time period compared to the present (Jahren et al. 1998).

In the present study, samples of comparable age to those of Jahren et al. (1998) are from the Bull Creek and Ravenscroft sites, both with an age of 10,000 cal B.P. The mean $\delta^{13}C$ value of the bone collagen from the Ravenscroft site is -11.57 ‰, which is more enriched than the Jahren et al. (1998) values. The mean $\delta^{13}C$ values of the bone and enamel hydroxyapatite from the Bull Creek and Ravenscroft sites are -2.28 ‰ and 0.17 ‰, respectively, which are also more enriched than the Jahren et al. (1998) values (Figure 31). The mean $\delta^{18}O$ values of the bone and enamel hydroxyapatite from the present study are -6.35 ‰ and -3.10 ‰, respectively, which are again more enriched than the values from Jahren et al. (1998) (Figure 32). The enrichment of the $\delta^{13}C$ and $\delta^{18}O$ values in the present study as compared to the Jahren et al. (1998) study could be due to a difference in the ages of the samples, as well as the differences in location. Samples from the present study that are older than 10,700 cal B.P. have less enriched $\delta^{13}C$ and $\delta^{18}O$ values, meaning as climate conditions warmed after the Younger Dryas, $\delta^{13}C$ and $\delta^{18}O$ values became more enriched toward 10,000 cal B.P. The Bull Creek and Ravenscroft sites are located at lower latitudes than the Hudson-Meng Bonebed site and precipitation at lower latitudes is more enriched in $\delta^{18}O$.

Connin et al. (1997) looked at bison teeth from the southwestern United States across a large timeline. Isotopic measurements that can be applied to this present study can be divided into four different groups based on site location. Fossil sites include: Murray Springs and Naco in southern Arizona, Dry Cave in southern New Mexico, and Blackwater Draw in eastern New Mexico. The mean $\delta^{13}C$ and $\delta^{18}O$ (in VSMOW) values from Murray Springs (10,850 $^{14}C$ yr B.P. or 12,700 cal B.P.) are 1.20 ‰ and 27.30 ‰ (-3.45 ‰, VPDB), respectively. These samples fall within the Younger Dryas.

However, the values are slightly more enriched compared to the present study, possibly because Murray Springs is located at a lower latitude, thus a higher temperature and an abundance of $C_4$ plants. The mean $\delta^{13}C$ and $\delta^{18}O$ (in VSMOW) values from Naco (11,000 $^{14}C$ yr B.P. or 12,850 cal B.P.) are 0.60 ‰ and 30.70 ‰ (-0.16 ‰, VPDB), respectively. These values are slightly more enriched than the present study, most likely for the same reason as the Murray Springs samples. The mean $\delta^{13}C$ and $\delta^{18}O$ (in VSMOW) values from Dry Cave (10,700 $^{14}C$ yr B.P. or 12,600 cal B.P.) are -2.80 ‰ and 24.10 ‰ (-6.56 ‰, VPDB), respectively. These samples are also within the Younger Dryas and are consistent with the values of the present study. Blackwater Draw has samples with ages ranging from 800 to 12,250 $^{14}C$ yr B.P. (700 to 14,100 cal B.P.) and mean values of $\delta^{13}C$ and $\delta^{18}O$ (in VSMOW) ranging from -1.30 ‰ to 1.10 ‰ and 24.60 ‰ to 28.9 ‰ (-6.07 ‰ to -1.90 ‰), respectively. The $\delta^{13}C$ values are also fairly consistent and follow the same pattern as the values from the present study (Figure 31). However, the $\delta^{18}O$ values vary slightly compared to the present study most likely due to the differences in latitudes for the locations of the sites and/or variability in the stable oxygen isotope composition of local water sources (Figure 32).

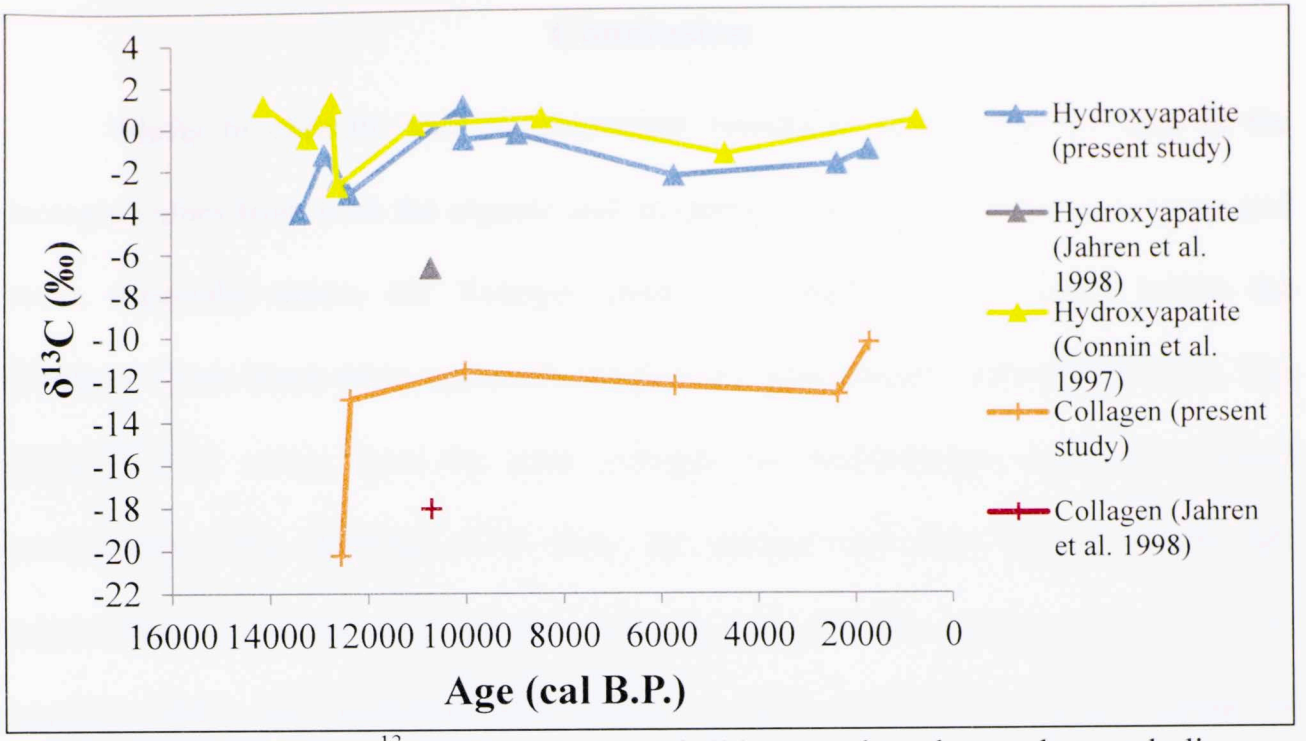


**Figure 31**. Average $\delta^{13}C$ values per site of all bone and tooth samples, excluding unreliable values, separated into hydroxyapatite (triangles) and collagen (crosses) for the present study (aqua and orange), the Jahren et al. 1998 study (gray and magenta), and the Connin et al. 1997 study (yellow).

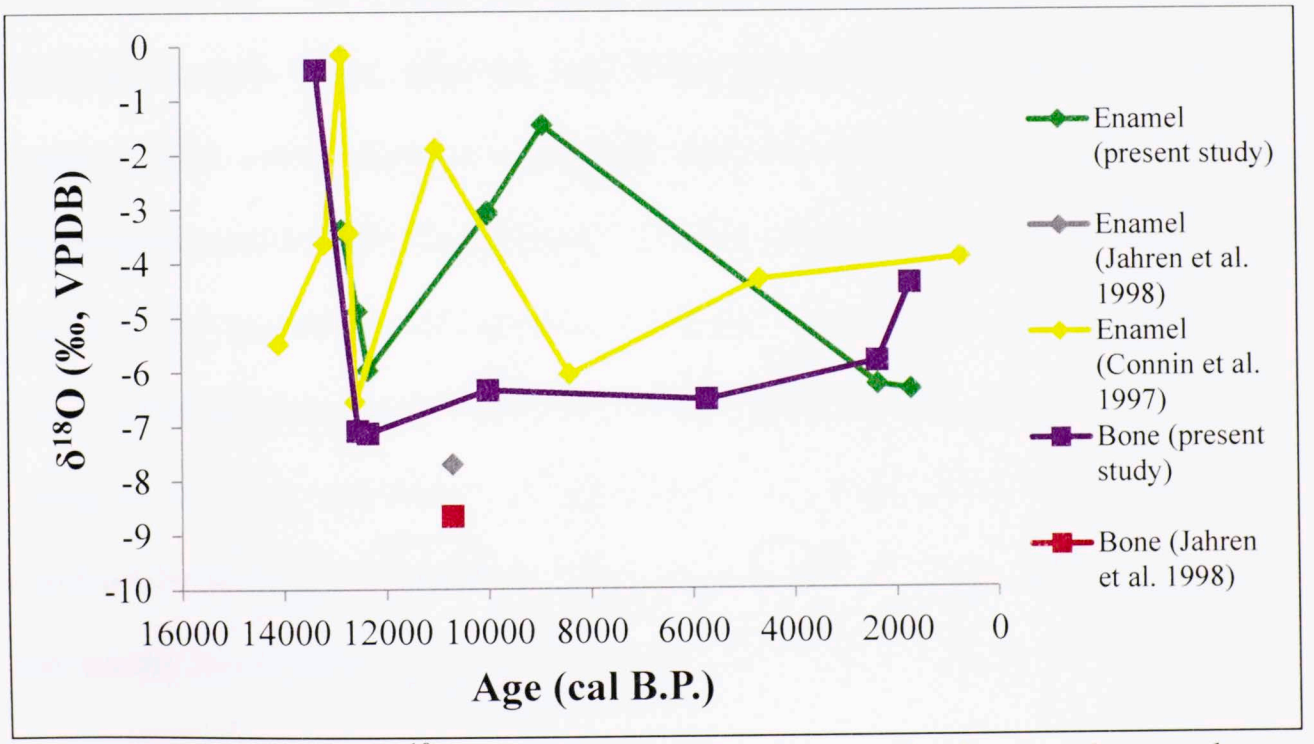


**Figure 32.** Average $\delta^{18}O$ values per site of hydroxyapatite from tooth enamel (diamonds) and bone samples (squares) for the present study (green and purple), the Jahren et al. 1998 study (gray and magenta), and the Connin et al. 1997 study (yellow).

## Conclusion

Shifts in climatic and environmental conditions can clearly be seen in the isotopic values from both the organic and inorganic components of the bison bones and teeth, especially across the Younger Dryas. The depleted $\delta^{13}C$ values within the Younger Dryas bison bone and teeth samples are representative of a $C_3$-rich diet. The depleted $\delta^{15}N$ values from the bone collagen are representative of a more humid environment. The depleted $\delta^{18}O$ from the enamel and bone hydroxyapatite are representative of cooler temperatures during this period. The isotopic values from the samples within the Younger Dryas indicate a shift to a cooler, wetter climate in Oklahoma. Although the Younger Dryas was overall a period of cooler, drier climatic conditions, some areas show wetter conditions (Fiedel 2011 and references therein), as seen in this study. This is likely due to the shift in air circulation patterns (Fiedel 2011). Enriched isotopic values after the end of the Younger Dryas indicate a shift to a warmer, drier environment as interpreted from the $C_4$-rich bison diet. Successful interpretations of climatic change based on fossil remains are highly dependent on the recovery and analysis of well-preserved materials. The amino acid distributions for many of the samples in the present study indicate good to excellent preservation of bone collagen, and thus vegetation and paleoclimate interpretation based on its isotopic composition is likely to be reliable. Future studies of the Younger Dryas in Oklahoma will greatly benefit from additional fossil bison materials as they become available.

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