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Increasing accuracy for the radiocarbon dating of sites occupied by the first Americans

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Abstract

Genetic analysis of Paleoamerican human remains suggests that people first entered the Americas
sometime between ~14,000 and ~16,000 years ago. Evaluation of these data requires unequivocal
archaeological evidence in a solid geological context that is well dated. Accurately determining the
age of late Pleistocene sites is thus crucial in explaining when and how humans colonized the
Americas. There are, however, significant challenges to dating reliability, especially when vertebrate
fossils (i.e. bones, teeth and ivory) are often the only datable materials preserved at sites.

We re-dated vertebrate fossils associated with the North American butchering sites of Wally's Beach
(Canada), La Prele [also known as Fetterman (Wyoming)], Lindsay (Montana), and Dent (Colorado).

Our work illustrates the crucial importance of sample chemical preparation in completely removing
contaminants derived from sediments or museum curation. Specifically, our work demonstrates that

chromatographic methods, e.g. preparative High Performance Liquid Chromatography and column chromatography using XAD resins, are currently the only efficient methods for removing environmental and museum-derived contaminants. These advanced techniques yield demonstrably more accurate AMS ^{14}C measurements that refine the ages of these four sites and thereby contribute to advancing our understanding of human dispersals across North America during the late Pleistocene.

Keywords

AMS Radiocarbon dating, preparative HPLC, hydroxyproline, XAD resin, Clovis complex, Pre-Clovis

1. Introduction

The arrival time of the first humans into North America is an extremely debated topic within the scientific community (Goebel et al., 2008; Meltzer, 2009, 2015). For most of the 20th century, it was widely believed that near the end of the last Ice Age, when sea levels were lower, prehistoric hunters from eastern Siberia followed prey animals across the Beringia Land Bridge into modern-day Alaska. When the ice sheets receded and exposed a path southward, the colonizers moved across the vast unpopulated continent, established a permanent human presence and, while doing so, possibly caused the extinction of 30+ genera of large mammals (Grayson and Meltzer, 2003; Haynes, 2013; Martin, 1958; Martin, 1973). These presumed earliest settlers were termed “Clovis”, a name derived from the town of Clovis, New Mexico, where their distinctive tools, dating to ca. 13,000 Cal BP, were first recognized at the site of Blackwater Draw (Waters and Stafford, 2007). The ensuing discovery of Clovis stone and osseous tools across North America reinforced the idea that Clovis people were the first Americans (Meltzer, 2009; Waters and Stafford, 2007). The discovery of new archaeological sites and the re-evaluation of old collections, however, suggest that humans reached the Americas several millennia before 13,000 Cal BP—the earliest time range for the Clovis complex (Amick,

2017; Bourgeon et al., 2017; Halligan et al., 2016; Waters and Stafford, 2007, 2014). Radiocarbon dates on organic matter from the archaeological site of Monte Verde, Chile, for example, point to a human occupation aged at around 12,300 BP or 14,200 Cal BP (MV-II).

To build robust chronologies for the peopling of the Americas, accurate radiocarbon dating is required. For radiocarbon results to be accurate, however, samples must be free of contamination. In this paper, we focus on the dating of collagen from vertebrate fossils that are commonly contaminated with humates accumulated during burial and/or preservatives added by museum curation processes. Humates were identified as primary contaminants in the early 1950s (Münnich, 1957), while post-excavation conservation was addressed somewhat later (Bronk Ramsey, 2008).

Inaccurate radiocarbon dates are rarely caused by problems associated with the measurement, but are predominately the result of inadequate sample pretreatment. Over the last 35 years, numerous methods have been used to chemically purify bone, teeth, and ivory for ^{14}C dating by accelerator mass spectrometry (AMS). At the Oxford Radiocarbon Accelerator Unit (ORAU), the most commonly used pre-treatment for bone samples is demineralization in HCl, followed by an alkali wash, and gelatinization followed by ultrafiltration (Brock et al., 2010a; Higham et al., 2006). In some cases, this method is unable to completely isolate uncontaminated collagen because of cross-linking and degradation of the collagen molecule. This is particularly true when samples are heavily contaminated with humic substances, conservation materials, or both. To resolve this problem, a few laboratories have used an entirely different approach—chromatography—to isolate the compound of interest. Following the work of Abelson and Hoering, who used ion exchange chromatography to isolate individual amino acids for stable isotope analysis (Abelson and Hoering, 1961), Ho *et al.* used cation exchange chromatography to purify petroleum-contaminated bones (Ho et al., 1969). They were followed by groups isolating a specific amino acid, hydroxyproline (HYP), for direct radiocarbon dating (Benders, 2010; Gillespie et al., 1984; Marom et al., 2013; Marom et al., 2012; Stafford et al., 1982; Stafford et al., 1991). Reverse phase chromatography is another chromatographic technique that separates humates from collagen hydrolyzates by using XAD resins (Stafford et al., 1988; Stafford et al., 1987). There are different types of XAD resins that are commercially available and are

described in (Stafford et al., 1988). They can be used to isolate weakly or non-ionized aliphatic and aromatic molecules from aqueous solutions. They are used, for example, to extract dilute organic chemicals from environmental and physiological fluids, to concentrate humates from fresh and marine waters, and in liquid chromatography, to separate weak polar compounds from aqueous solutions (Stafford et al., 1988). The first application of this approach for ^{14}C dating archaeological bones was by Stafford *et al.* in 1982 and 1988 (Stafford et al., 1988; Stafford et al., 1982). They developed this procedure to remove humates from gelatin hydrolyzates for either subsequent isolation of hydroxyproline and proline or in the dating the XAD-purified hydrolyzate directly (Stafford et al., 1988; Stafford et al., 1982). A flow chart showing the different fractions that can be isolated using this method is reported in Figure 1. Subsequently, the method has been applied to numerous archaeological and paleontological dating projects, including samples from North and South America (Waters and Stafford, 2007; Waters et al., 2015).

At the ORAU, attention has focused on dating the amino acid hydroxyproline, which is obtained after the hydrolysis of collagen and separation by preparative High Performance Liquid Chromatography (prep-HPLC) (Devièse et al., 2018; Gillespie et al., 1986; Gillespie et al., 1984; Marom et al., 2012; Nalawade-Chavan et al., 2014). A flow chart showing the different steps of the procedure is reported in Figure 2. The efficiency of the method in removing contaminants from heavily contaminated samples and its ability to provide very accurate ^{14}C measurements has been demonstrated in several recent notable cases involving Paleolithic sites in France (Bourrillon et al., 2017), Croatia (Devièse et al., 2017), Russia (Marom et al., 2012; Nalawade-Chavan et al., 2014; Reynolds et al., 2017; Sikora et al., 2017) and the Americas (Becerra-Valdivia et al., 2018).

Accurate AMS ^{14}C dates on fossil bone are crucial to testing archaeological and paleontological hypotheses. In this paper, we evaluate the accuracy of two important chemical purification methods: isolating HYP by prep-HPLC, and using XAD resins to purify collagen hydrolysates. To do this, we chose four North American archaeological sites that had already been dated by XAD resin methods and we re-dated the same bone specimens by extracting hydroxyproline. We also compare the results against other ^{14}C determinations obtained using different pretreatment methods.

2. Materials

There are multiple Clovis-aged and older archaeological sites in North America where human presence is established by lithic assemblages, taphonomy, cut marks or combinations of these. For our experiment, seven vertebrate fossils associated with the North American butchering sites of Wally's Beach (Canada), La Prele [also known as Fetterman (Wyoming)], Lindsay (Montana) and Dent (Colorado) were selected (Table 1).

Three bone samples included in this study are from Wally's Beach (Table 1). This site is located at St. Mary's Reservoir, Alberta (Canada), and represents the only known late Pleistocene kill and butchery site at the southern margin of the ice-free corridor (Kooyman et al., 2006; Kooyman et al., 2012; Waters et al., 2015). The animal assemblage includes extinct megafauna (camel and horse), extinct muskox (*Bootherium bombifrons*), caribou and bison. The animal remains are being exposed by eolian deflation, but were originally buried by 1.5 to 2.0 m of eolian silt and sand that overlies Wisconsinan glacio-fluvial sediments. At the site, seven horses and one camel were killed and butchered by humans based on cut marks on bones and the partial scattering and dismemberment of the carcasses. Each of the carcasses was horizontally separated from one another by 25-100 m over a distance of 500 m and were found with non-diagnostic lithic artifacts (Waters et al., 2015). Two samples are from Dent, Colorado (USA). This site was originally discovered in 1932 when flood runoff uncovered a mammoth bone near Milliken, Colorado (Brunswig, 2007). The initial excavation in 1932 revealed fluted Clovis projectile points among the bones (Brunswig, 2007). Subsequent excavations revealed the presence of 15 individual mammoths (Saunders, 1999) within a bone stratum 1.5 m thick. Brunswig considers the site to represent humans killing a mammoth herd based on the number and position of projectile points recovered among the bones. Both samples are from mammoth (*Mammuthus columbi*) elements and contain humic acid contamination. One of the two samples was also preserved with an unknown adhesive, possibly Gelva (Table 1).

Another mammoth sample selected for this study is from the La Prele site, Wyoming, USA (formerly called the Fetterman site). Excavations at the site in 1986 produced a single sub-adult mammoth (*Mammuthus columbi*), the fragmental remains of a bison (*Bison* sp.) and assorted lithic artifacts (Byers, 2002). The mammoth was found 27 cm below the surface of soil 4, which was colluvium and described as a “massive clayey sand” (Byers, 2002). Excavations during the last few years have also produced artifacts of the Clovis complex (Byers, 2002; Mackie et al., 2017). The bones were deemed physically unstable and were stabilized and reconstructed using adhesives, including Glyptol and Paraloid B-72. A neural spine unquestionably from the mammoth was selected for dating (Table 1). The final specimen was from a mammoth (*Mammuthus columbi*) that was excavated in 1967 from late Pleistocene loess near Lindsay, Montana, USA (Davis and Wilson, 1985). At this site, loess began to accumulate at the end of the Pleistocene and was derived from a nearby glacial lake bed that was a few kilometers from the Wisconsin maximum ice margin (Davis and Wilson, 1985). The Lindsay mammoth was interpreted as a cultural site based on the taphonomic patterns of disarticulation and spiral fracturing (Davis and Wilson, 1985; Hill and Davis, 1998; Hill and Davis, 2014; Waters and Stafford, 2014). The absence of lithic artifacts was used to question the site’s human presence (Grayson and Meltzer, 2015). However, based on taphonomic analyses by Krasinski, the bones showed evidence of butchering with stone tools. Cut marks throughout the skeleton suggests disarticulation and meat stripping (Krasinski, 2010). Eight sandstone blocks (boulders) found underneath several bones further support the hypothesis of a butchery site (Davis and Wilson, 1985; Krasinski, 2010; Waters and Stafford, 2014). The sandstone blocks are believed to be manuports used to crack open the bones for marrow extraction. The final fossil included in this study is a rib from a gray whale (*Eschrichtius robustus*), a chemically and physically well-preserved bone used as a background reference sample (Table 1). It was excavated from a site 20 km south of the Beaufort Sea coast in Alaska (USA). It had been preserved in permafrost, dates >70,000 BP and is therefore significantly beyond the 55 ka limit of the radiocarbon dating method (Stafford et al., 1987).

Table 1: Description of North American fossil bones dated using the single amino acid method at the ORAU. P, ORAU accession number; SR, Stafford Research lab number. Sample SR-8221 had to be resampled for additional radiocarbon measurements and was given a different P number.

ORAU P Number	SR Number	Site	Taxon	Contaminant
P39336	SR-5156	Beaufort Sea, Alaska, USA	<i>Eschrichtius robustus</i>	None identified
P39331	SR-8171	Wally's Beach, Alberta, Canada	<i>Camelops hesternus</i>	Humic acids
P39332	SR-8226	Wally's Beach, Alberta, Canada	<i>Bootherium bombifrons</i>	Humic acids
P39333 P45701	SR-8221	Wally's Beach, Alberta, Canada	<i>Equus conversidens</i>	Butvar B-98
P39334	SR-7616	Dent, Colorado, USA	<i>Mammuthus columbi</i>	Humic acids
P39335	SR-6606	Dent, Colorado, USA	<i>Mammuthus columbi</i>	Unknown adhesive
P39337	SR-7356	La Prele, Wyoming, USA	<i>Mammuthus columbi</i>	Paraloid B-72 & Glyptol
P39338	SR-8253	Lindsay, Montana, USA	<i>Mammuthus columbi</i>	Unknown adhesive

3. Methods

3.1 Percent Nitrogen (%N)

Before any chemical treatment was started, we tested collagen content by removing 3 to 5 mg of bone powder with an electrical drill with a tungsten carbide drill bit and measured the %C, %N and atomic C/N ratio of the bone powder. The method used an automated carbon and nitrogen elemental analyzer (Carlo Erba EA1108) coupled with a continuous-flow isotope monitoring mass spectrometer (Europa Geo 20/20).

3.2 XAD procedure for radiocarbon dating

All samples dated following XAD purification have been prepared using the procedure described in (Waters et al., 2015). Figure 1 shows a flow chart with the different steps used by Tom Stafford, and the different fractions that can be separated for AMS dating.

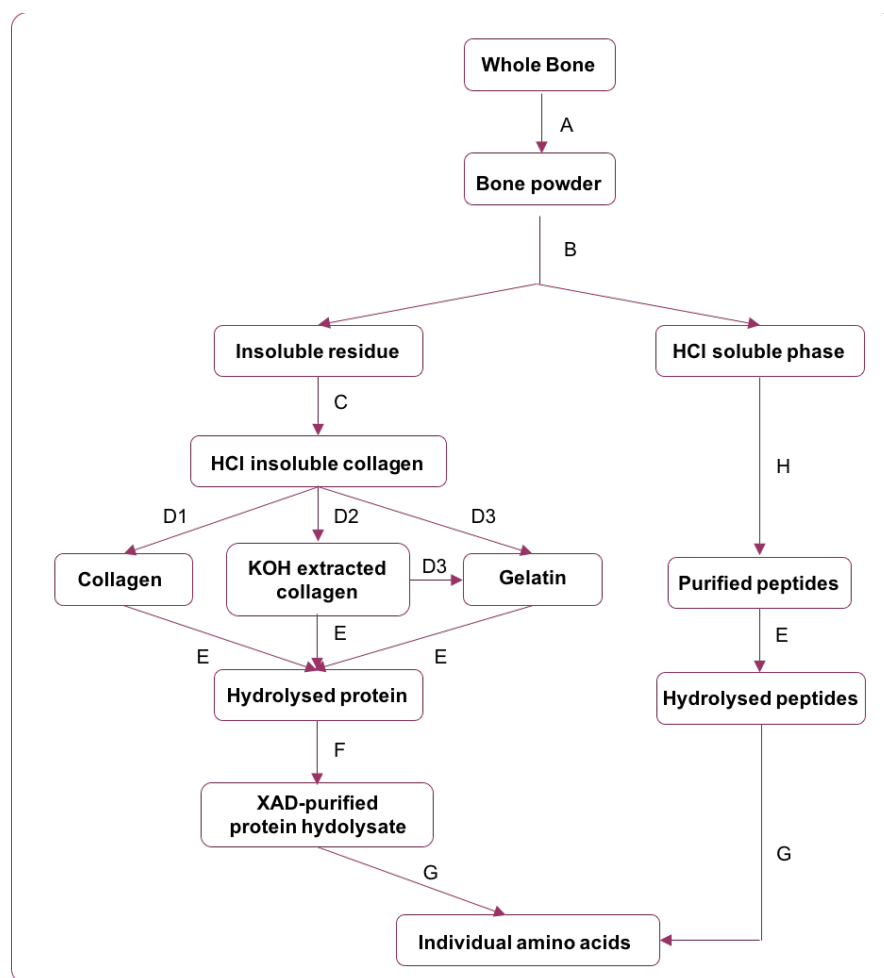


Figure 1: Flow diagram showing pretreatment methods used by Tom Stafford and discussed in this article. This diagram is based on (Stafford et al., 1987; Waters et al., 2015). **A:** Physical cleaning and grinding ; **B:** Decalcification with 0.6 N HCl; **C:** Water washes; **D1:** Lyophilisation; **D2:** KOH extraction; **D3:** Gelatinisation; **E:** Hydrolysis (6 N HCl at 110°C for 24 hr.); **F:** Purification on XAD resin; **G:** HPLC separation; **H:** Filtration and Reverse Phase Chromatography.

3.3 Radiocarbon dating of bones at the ORAU

Samples were first pre-treated following the routine procedure at the ORAU comprising decalcification in acid, a base wash, re-acidification, gelatinisation and ultrafiltration (coded 'AF '), as described in (Brock et al., 2010a) and illustrated on Fig. 2. Samples that had been preserved with glues or those contaminated by humics were washed with organic solvents (acetone, methanol and

chloroform) prior to AF treatment (coded 'AF*'). Samples were also re-dated using the single amino acid radiocarbon dating method optimised at ORAU (coded 'HYP'). Freeze-dried collagen samples (40-50 mg) were hydrolysed using 6M hydrochloric acid and hydroxyproline separated on a Varian ProStar HPLC system following the procedure detailed in (Devièse et al., 2018) and illustrated on Fig. 2.. Collagen or hydroxyproline samples were then combusted, graphitised and dated by AMS following the procedure as described in (Brock et al., 2010a).

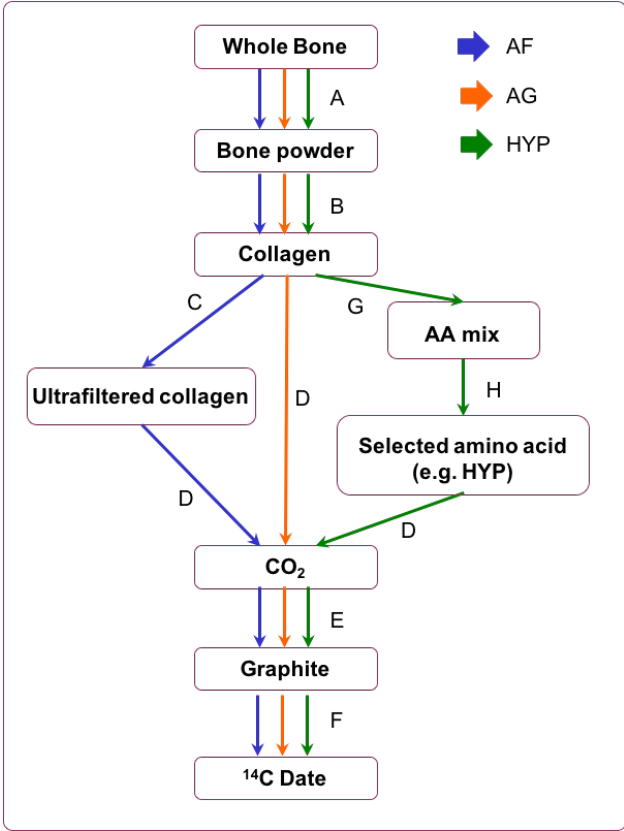


Figure 2: Flow diagram showing pretreatment methods used at the ORAU and discussed in this article. **A:** Physical cleaning and grinding; **B:** Acid – Base – Acid treatment (ABA); **C:** Ultrafiltration; **D:** Combustion; **E:** Graphitization; **F:** AMS measurement; **G:** hydrolysis; **H:** Prep-HPLC. Technical details for each method can be found in (Brock et al., 2010a) and (Devièse et al., 2018)

4. Results

4.1 Percent Nitrogen

Percent total nitrogen (%N) of whole bone, used as an indicator of collagen preservation, was measured for each fossil bone. All values were > 0.75% (Table 2), the threshold for accepting bones for AMS ^{14}C dating at ORAU (Brock et al., 2010b). Importantly, all samples had significantly different %N values. This can be attributed to variable collagen preservation in the bone, the presence of nitrogen containing contaminants, or both. Sample P39336, selected as a background reference sample in this study, was already known to have good collagen preservation. Unsurprisingly, its %N is higher than all the other samples and is in the range of values expected for modern bone ($4.0 < \%N < 4.5$).

Table 2: Total %N data for the 8 whole bone samples. To be acceptable, bone samples must have %N values >0.75% at the ORAU. For this study, the 8 samples passed the %N test.

Oxford P Number	Sample Number	Site	Taxon	%N
P39336	SR-5156	Beaufort Sea, Alaska, USA	<i>Eschrichtius robustus</i>	4.08
P39331	SR-8171	Wally's Beach, Alberta, Canada	<i>Camelops hesternus</i>	0.94
P39332	SR-8226	Wally's Beach, Alberta, Canada	<i>Bootherium bombifrons</i>	2.09
P39333	SR-8221	Wally's Beach, Alberta, Canada	<i>Equus conversidens</i>	1.17
P39334	SR-7616	Dent, Colorado, USA	<i>Mammuthus columbi</i>	1.57
P39335	SR-6606	Dent, Colorado, USA	<i>Mammuthus columbi</i>	0.76
P39337	SR-7356	La Prele, Wyoming, USA	<i>Mammuthus columbi</i>	0.87
P39338	SR-8253	Lindsay, Montana, USA	<i>Mammuthus columbi</i>	0.92

4.2 Comparison of dates obtained on collagen (AF/AF*) and on Hydroxyproline (HYP)

Collagen was extracted from the 8 bones included in this study in order to obtain a radiocarbon date on bulk collagen after ultrafiltration and another one on hydroxyproline isolated by preparative HPLC. The new chronometric data are reported reported in Table 3. The Beaufort Sea bone, used as a background standard in this study, produced dates beyond the radiocarbon limit using both protocols, as expected. For the other seven samples, Chi-squared tests were run on paired AF/AF* and HYP dates using their modern carbon fractions (Table 4). In three of the cases, the dates obtained on ultrafiltered collagen and hydroxyproline are not statistically distinguishable, indicating either that all the contaminant had been removed by the AF/AF* treatment, or that there is no significant contamination present in the bones. In the other four cases, the paired dates failed the Chi-squared test. For sample SR-8221, the date obtained on collagen is slightly older than the date obtained on hydroxyproline. It is important to note here that this specimen had to be resampled for the AF treatment while, for the other 7 specimens, the two treatments were performed on the exact same sample. For the 3 other pairs that failed the Chi-squared test, the dates obtained on hydroxyproline are older than those obtained on collagen. For these samples, we suspect that some contaminant had remained in the collagen (possibly crosslinked to it) but was removed by hydrolysing the collagen and isolating hydroxyproline. Ages obtained on hydroxyproline are therefore retained over those obtained from bulk collagen for the following sections of the paper.

241 **Table 3:** Radiocarbon determinations and analytical data for the eight late Pleistocene to early Holocene bones from North America used in this
 242 study and dated at the Oxford Radiocarbon Accelerator Unit (ORAU). PCode refers to pretreatment code; 'AF' is ultrafiltered collagen; 'HYP'
 243 denotes the extraction of hydroxyproline from hydrolysed bone collagen. Samples that had been preserved with glues were also washed with
 244 solvents (acetone, methanol and chloroform) prior to AF treatment (coded 'AF*'). CRA is conventional radiocarbon age, expressed in years BP
 245 with 1 σ standard deviation (Stuiver and Polach, 1977). Collagen yield is based on the mass of gelatin versus mass of bone powder
 246 demineralized. Mass of Hyp is estimated by the peak area on the chromatogram. Stable isotope ratios are expressed in per mil (‰) relative to
 247 VPDB with a mass spectrometric precision of $\pm 0.2\%$ (Coplen Tyler, 1994). C/N is the atomic ratio of carbon to nitrogen and is acceptable if it
 248 ranges between 2.9-3.5 in the case of collagen, or ~ 5.0 in the case of hydroxyproline (Brock et al., 2010a; Devière et al., 2018).
 249

SITE	P Number	P code	CRA ($\pm 1 \sigma$ SD)	AMS LAB No. OxA-X	Bone (mg)	Collagen (mg)	Collagen Yield %	Mass collagen hydrolysed (mg)	HYP (mg)	$\delta^{13}\text{C}$ (‰) (VPDB)	$\delta^{15}\text{N}$ (‰) (AIR)	C/N (Atomic %)
Beaufort (Whale)	P39336.0	AF	> 49,900	OxA-36957	1010	26.06	2.6	/	/	-14.3	14.0	3.2
	P39336.0	HYP	> 50,000	OxA-X-2736-13	1010	126.36	12.5	47.3	3.2	-19.4	25.8	5.1
Wally's Beach (Camelops)	P39331.1	AF*	11,530 $\pm$ 55	OxA-36953	2000	33.25	1.7	/	/	-19.4	2.7	3.2
	P39331.1	HYP	11,530 $\pm$ 50	OxA-X-2736-8	2000	94.17	4.7	48.5	3.6	-18.4	16.6	4.9
Wally's Beach (Bootherium)	P39332.0	AF*	11,295 $\pm$ 50	OxA-36954	2030	19.52	1	/	/	-19.4	1.5	3.2
	P39332.1	HYP	11,255 $\pm$ 50	OxA-X-2736-9	1140	70.63	6.2	48.8	4.5	-21.9	11.0	4.9
Wally's Beach	P45701.0	AF*	11,685 $\pm$ 50	OxA-37337	867	28.24	3.3	/	/	-21.2	-0.6	3.4

<i>(Equus)</i>	P39333.2	HYP	11,445 ± 55	OxA-X-2736-10	880	39.62	4.5	39.6	3.5	-23.3	11.9	5.0
Dent <i>(Mammuthus)</i>	P39334.1	AF*	11,115 ± 50	OxA-36955	2100	28.61	1.4	/	/	-10.9	11.2	3.2
	P39334.1	HYP	11,055 ± 50	OxA-X-2736-11	2100	98.14	4.7	47.2	4.1	-13.6	18.4	5.0
Dent <i>(Mammuthus)</i>	P39335.2	AF*	10,870 ± 50	OxA-36956	796	20.14	2.5	/	/	-14.0	8.9	3.3
	P39335.1	HYP	11,155 ± 50	OxA-X-2736-12	1610	51.9	3.2	48.4	4.3	-18.0	14.7	5.0
La Prele <i>(Mammuthus)</i>	P39337.1	AF*	9,320 ± 45	OxA-36958	1380	38.73	2.8	/	/	-19.5	7.0	3.2
	P39337.1	HYP	11,035 ± 50	OxA-X-2736-14	1380	163.96	11.9	49.2	4.9	-22.7	13.0	4.9
Lindsay <i>(Mammuthus)</i>	P39338.2	AF*	11,720 ± 60	OxA-37113	1140	13.71	1.2	/	/	-20.7	3.8	3.3
	P39338.2	HYP	12,395 ± 55	OxA-X-2736-15	1140	66.13	5.8	48.6	4.4	-24.9	9.7	5.0

Table 4: Chi-squared test results on radiocarbon dates obtained using the UF/UF* or HYP protocols. The error weighted mean and the t values were calculated for each pair of samples using the AMS modern fraction values. If t is < 3.84, the error weighted mean is not significant and the 2 dates are therefore statistically identical.

Sample Numbers	AF/AF* dates	Hyp Dates	Error-weighted-means	Chi squared results
SR-5156	> 49,900 (OxA-36957)	> 50,000 (OxA-X-2736-13)	/	/
SR-8171	11,530 ± 55 (OxA-36953)	11,530 ± 50 (OxA-X-2736-8)	0.2381 ± 0.0110	Pass (t=0.00)
SR-8226	11,295 ± 50 (OxA-36954)	11,255 ± 50 (OxA-X-2736-9)	0.2458 ± 0.0011	Pass (t=0.33)
SR-8221	11,685 ± 50 (OxA-37337)	11,445 ± 55 (OxA-X-2736-10)	0.2368 ± 0.0011	Fail (t=10.43)
SR-7616	11,115 ± 50 (OxA-36955)	11,055 ± 50 (OxA-X-2736-11)	0.2515 ± 0.0011	Pass (t=0.75)
SR-6606	10,870 ± 50 (OxA-36956)	11,155 ± 50 (OxA-X-2736-12)	0.2539 ± 0.0011	Fail (t=16.46)
SR-7356	9,320 ± 45 (OxA-36958)	11,035 ± 50 (OxA-X-2736-14)	0.2823 ± 0.0012	Fail (t=658.89)
SR-8253	11,720 ± 60 (OxA-37113)	12,395 ± 55 (OxA-X-2736-15)	0.2220 ± 0.0011	Fail (t=70.04)

4.2.1 Beaufort Sea Coast Whale, Alaska, USA

The age of the sample from the Beaufort Sea, based on its stratigraphic position, is beyond the radiocarbon dating limit. Previous attempts to date the sample with different methods have provided greater than radiocarbon background ages but, with seemingly poor laboratory backgrounds, the minimum ages are very young (Table 5) (Stafford et al., 1987). More recently, Stafford obtained an age of 45,580 ± 270 BP after purification of the hydrolysed collagen using XAD resin (Stafford, 2014). We re-dated the same sample using the AF and HYP procedures. We obtained measurements of >49,900 years (OxA-36957) and >50,000 years (OxA-X-2736-13), respectively, which indicates they date to beyond the radiocarbon limit as expected for this sample.

Table 5: Previously published dates for the sample from Beaufort Sea, Alaska, USA. Lab codes are AA for University of Arizona Accelerator Mass Spectrometry Laboratory; UCIAMS for University of California-Irvine W.M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory.

Sample Number	Sample Preparation	^{14}C y BP (1 σ)	References
AA-312A	HCl insoluble collagen (A+B+C, Fig. 1)	>26,700	(Stafford et al., 1987)
AA-312B	Gelatin (A+B+C+D3, Fig. 1)	>27,300	(Stafford et al., 1987)
AA-312C	Gelatin (A+B+C+D3, Fig. 1)	>38,000	(Stafford et al., 1987)
UCIAMS-116378	XAD purified hydrolysed protein (A+B+C+D3+ E +F, Fig. 1)	45,580 $\pm$ 270	(Stafford, 2014)

4.2.2 Wally's Beach, Alberta, Canada

The Wally's Beach site was initially dated by five radiocarbon ages ranging from 10,980 $\pm$ 80 BP (TO-7691) to 11,350 $\pm$ 80 BP (TO-8972) from bison, horse, muskox, caribou and camel bones from the eolian sediments (Kooyman et al., 2006; Kooyman et al., 2012; Waters et al., 2015). These ages were based on the unpurified gelatin fraction (Table 6). Twenty-seven radiocarbon ages were also obtained on XAD-purified collagen and other chemical fractions extracted from bones of all seven butchered horses, the butchered camel, and the un-butchered muskox (Waters et al., 2015). The eight XAD-purified collagen dates for the horses overlap at 1 σ and range from 11,410 $\pm$ 30 BP (UCIAMS-127349) to 11,470 $\pm$ 35 BP (UCIAMS-127348). The XAD-purified collagen from the camel bone yielded two ages [11,465 $\pm$ 40 BP (UCIAMS-116400) and 11,425 $\pm$ 30 BP (UCIAMS-127347)] that are statistically identical. The initial gelatin radiocarbon age of 10,980 $\pm$ 80 BP (TO-7691) for the muskox was revised to 11,320 $\pm$ 30 BP (UCIAMS-12737) using XAD collagen.

For this new study, three samples were selected for dating using the HYP method: the camel bone (*Camelops hesternus*), the muskox bone (*Bootherium bombifrons*) and one of the butchered horse bone (*Equus conversidens*; Horse A). Artifacts were found with the camel and the horse but no artifact was found with the unbutchered muskox. All samples were contaminated with humates; the horse bone was also coated with Butvar B-98 resin (Waters et al., 2015). Using the single amino acid dating approach, the *Camelops hesternus* (P39331) was dated at 11,530 $\pm$ 50 BP (OxA-X-2736-8), the *Bootherium bombifrons* (P39332) at 11,255 $\pm$ 50 BP (OxA-X-2736-9), and the *Equus*

conversidens (P39333) at $11,445 \pm 55$ BP (OxA-X-2736-10). We performed a Chi-squared test on these three new dates using the modern carbon fraction ($F^{14}C$) and its error. The error-weighted-mean in $F^{14}C$ was 0.2415 ± 0.0009 . The t value calculated for the Chi-squared test is 15.97. For a Chi-squared test with 3 values, if t is > 5.99 the error is significant. This test therefore shows that the results obtained on the three bones samples from Wally's Beach are not contemporaneous. The dates for the *Camelops hesternus* (P39331) and the *Equus conversidens* (P39333) are statistically the same but the date for the *Bootherium bombifrons* bone (P39332) is younger and therefore it appears that the unbutchered skeleton derives from a later, probably natural event. This is coherent with the ages obtained after XAD purification. From an archaeological perspective, the dates obtained on the *Camelops hesternus* (P39331) and the *Equus conversidens* (P39333) indicate that these animals were hunted some 300 years earlier than the earliest firmly dated Clovis site (Waters and Stafford, 2007).

Table 6: Previously published dates for the three samples from Wally's Beach, Alberta, Canada included in this study. Lab code TO denotes the IsoTrace Laboratory.

Sample Number	Sample Preparation	^{14}C y BP (1 σ)	References
<i>Camelops hesternus</i>			
Cat No. 3610.1			
TO-13513	Gelatin (A+B+C+D3, Fig. 1)	$11,070 \pm 80$	(Waters et al., 2015)
UCIAMS-116390	KOH extracted collagen (A+B+C+D2, Fig. 1)	$11,425 \pm 35$	(Waters et al., 2015)
UCIAMS-116383	Gelatin (A+B+C+D3, Fig. 1)	$11,420 \pm 30$	(Waters et al., 2015)
UCIAMS-116400	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	$11,465 \pm 40$	(Waters et al., 2015)
UCIAMS-127347	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	$11,425 \pm 30$	(Waters et al., 2015)
<i>Bootherium bombifrons</i>			
Cat No. 3293.1			
TO-7691	Gelatin (A+B+C+D3, Fig. 1)	$10,980 \pm 80$	(Waters et al., 2015)
UCIAMS-127371	KOH extracted collagen (A+B+C+D2, Fig. 1)	$11,170 \pm 30$	(Waters et al., 2015)
UCIAMS-127372	Gelatin (A+B+C+D3, Fig. 1)	$11,255 \pm 30$	(Waters et al., 2015)
UCIAMS-127373	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	$11,320 \pm 30$	(Waters et al., 2015)
<i>Equus conversidens</i>			
Horse A, Cat No. 315			
UCIAMS-127363	KOH extracted collagen (A+B+C+D2, Fig. 1)	$13,540 \pm 40$	(Waters et al., 2015)

UCIAMS-127364	Gelatin (A+B+C+D2+D3, Fig. 1)	11,495 ± 30	(Waters et al., 2015)
UCIAMS-127351	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	11,440 ± 30	(Waters et al., 2015)

305

306 4.2.3 Dent, Colorado, USA

307 The Dent site was also believed to have been a kill and butchery site that occurred as a single event.

308 There have been several attempts at dating the bones from the site (Table 7). The first radiocarbon

309 date was 7,200 ± 200 BP (I-473) on whole bone. However, the age was considered too young due to

310 shellac contamination. After solvent extraction to remove the shellac, the same bone yielded a ¹⁴C

311 measurement of 11,200 ± 500 BP (I-622), which Haynes et al. argue to be the correct age (Haynes,

312 1966; Haynes et al., 1998; Trautman and Willis, 1966). The first attempts to date hydrolysed collagen

313 purified by XAD produced dates ranging from 10,590 ± 500 BP (AA-832) to 10,980 ± 90 BP (AA-

314 2941). Dates obtained at the same time on single amino acids produced similar ages (Table 7). More

315 recently, three additional dates were produced on collagen purified by XAD and they are older than

316 most of the dates previously obtained (Table 7). This may indicate that there was some contaminant

317 not being fully removed from the samples but, unfortunately, there is no material left to check this. The

318 two new hydroxyproline dates obtained at the ORAU are 11,055 ± 50 BP (OxA-X-2736-11) and

319 11,155 ± 50 BP (OxA-X-2736-12). Using the modern carbon fraction of these two HYP dates, we ran

320 a Chi-squared test. The error weighted mean in F¹⁴C was 0.2510 ± 0.0011 and *t* = 1.90. This *t* value

321 is <3.84. The two HYP dates are therefore statistically indistinguishable and provide a narrower time

322 window within which these mammoths were killed.

323

324 **Table 7:** Previously published dates for the samples from Dent, Colorado USA. Lab code I stands for

325 Teledyne Isotopes.

Sample Number	Sample Preparation	¹⁴ C y BP (1 σ)	References
I-473	HCl insoluble collagen (A+B+C, Fig. 1)	7,200 ± 200	(Trautman and Willis, 1966)
I-622	HCl insoluble collagen (A+B+C, Fig. 1)	11,200 ± 500	(Trautman and Willis, 1966)
AA-830	HCl insoluble collagen (A+B+C, Fig. 1)	8,250 ± 520	(Brunswig, 2007; Stafford et al., 1988)
AA-831	Gelatin (A+B+C+D3, Fig. 1)	9,240 ± 350	(Stafford et al., 1988; Stafford et al., 1987)

AA-832	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,590 ± 500	(Stafford et al., 1988; Stafford et al., 1991)
AA-833	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,950 ± 480	(Stafford et al., 1988; Stafford et al., 1987)
AA-2941	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,980 ± 90	(Stafford et al., 1988; Stafford et al., 1991)
AA-2942	Asp – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,750 ± 170	(Stafford et al., 1991)
AA-2943	Glu – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,890 ± 110	(Stafford et al., 1991)
AA-2944	Thr – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,380 ± 140	(Stafford et al., 1991)
AA-2945	Hyp – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,680 ± 90	(Stafford et al., 1988; Stafford et al., 1991)
AA-2946	Gly – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,780 ± 90	(Stafford et al., 1991)
AA-2947	Ala – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,690 ± 120	(Stafford et al., 1991)
UCIAMS-11339	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	11,065 ± 35	(Waters and Stafford, 2007)
UCIAMS-11340	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,940 ± 30	(Waters and Stafford, 2007)
UCIAMS-116403	XAD purified hydrolysed protein (A+B+C+D3+E+F, Fig. 1)	10,960 ± 35	(Stafford, 2014)
UCIAMS-116394	KOH extracted collagen (A+B+C+D2, Fig. 1)	10,925 ± 35	(Stafford, 2014)
UCIAMS-116388	Gelatin (A+B+C+D2+D3, Fig. 1)	11,015 ± 30	(Stafford, 2014)

326

327 4.2.4 La Prele Mammoth (previously named Fetterman Mammoth), Wyoming, USA

328 The first ¹⁴C dating on the La Prele mammoth produced dates that were significantly younger than
329 Folsom or Clovis (Table 8). The fossil bone submitted as mammoth produced an early Holocene age
330 based on dating of two chemical fractions that overlapped at one standard deviation [9060 ± 50 BP
331 (CAMS-72350) and 8890 ± 60 BP (CAMS-74661) on KOH-collagen and gelatin, respectively] (Table
332 8). Three hypotheses were proposed to explain these Holocene ages; 1) the specimen represents a
333 relict (Holocene) mammoth population in that region; 2) the dated sample was contaminated with
334 modern carbon, possibly derived from a mixture of Glyptol and Paraloid B-72 (Byers, 2002) and; 3)
335 the first two AMS dates were on a nearby bison rather than the target mammoth. Stafford *et al.* re-
336 dated a different sample and used a neural spine that was unquestionably from the mammoth. Their
337 resulting date was 10,760 ± 30 BP (UCIAMS-40174) on gelatin and 10,965 ± 30 BP (UCIAMS-
338 206764) on XAD purified protein hydrolysate. This same neural spine was selected for dating by the
339 HYP method at the ORAU. After a solvent wash to remove the conservation material, we isolated
340 hydroxyproline and dated it. We obtained an age of 11,035 ± 50 BP (OxA-X-2736-14). These new
341 dates are significantly older than those published by (Byers, 2002) and allow us to confirm the Clovis
342 age attribution for this site.

343

344

345 **Table 8:** Previously dates for the samples from La Prele Mammoth, Wyoming, USA. Lab code CAMS
 346 is for Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, California.
 347 Although bone for CAMS-72350 and 74661 was submitted as *Mammuthus* sp., subsequent
 348 examination of the collections indicates the specimen was probably *Bison* sp. and not *Mammuthus*. A
 349 complete *Mammuthus* neural spine was located in the University of Wyoming collections and was
 350 used for all ^{14}C dates listed as *Mammuthus* sp. for the La Prele Mammoth.

Sample Number	Sample Preparation	^{14}C y BP (1 σ)	References
CAMS-72350	KOH extracted collagen (A+B+C+D2, Fig. 1)	9,060 $\pm$ 50	(Byers, 2002)
CAMS-74661	Gelatin (A+B+C+D3, Fig. 1)	8,890 $\pm$ 60	(Byers, 2002)
UCIAMS-40174	Gelatin (A+B+C+D2+D3, Fig. 1)	10,760 $\pm$ 30	This paper
UCIAMS-206764	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	10,965 $\pm$ 30	This paper

351

352 4.2.5 Lindsay, Montana, USA

353 Five radiocarbon dates on bone were initially obtained from the Lindsay mammoth (Davis and Wilson,
 354 1985; Hill and Davis, 1998; Hill, 2006; Huber and Hill, 2003). These dates are 9,490 $\pm$ 135 BP (I-
 355 7028), 10,700 $\pm$ 290 BP (WSU-652), 10,980 $\pm$ 225 BP (I-9220), 11,500 $\pm$ 80 BP (Beta-102031), and
 356 11,925 $\pm$ 350 BP (S-918). These ages were all derived from unpurified bone collagen and therefore
 357 must represent minimum ages. Subsequently, three additional dates were obtained on a mammoth rib
 358 from the site: KOH extracted collagen yielded an age of 12,105 $\pm$ 40 BP (CAMS-82416), gelatin
 359 yielded an age of 12,175 $\pm$ 40 BP (CAMS-80541), and the XAD-purified collagen yielded an age of
 360 12,330 $\pm$ 50 BP (CAMS-72348). To estimate the age of the Lindsay mammoth, Krasinski averaged all
 361 eight radiocarbon measurements—from those on unpurified and purified collagen and spanning 9,490
 362 to 12,330 BP—to derive an average age of 11,210 $\pm$ 190 BP or 12,920 to 13,300 Cal BP for the site.
 363 Based on this averaging method, she suggested that this butchering likely represented the work of
 364 Clovis hunters (Krasinski, 2010). Evaluation of the radiocarbon record indicates that the unpurified
 365 bone collagen ages are minimum ages and cannot be used for valid age interpretations. Based on the
 366 chemical fractions dated, the XAD-purified collagen that gave the age of 12,330 $\pm$ 40 BP or 14,140 to
 367 14,400 Cal BP (CAMS-72348) provided the most accurate age from this second group of dates. Six
 368 additional ages have been obtained on different chemical fractions from Lindsay mammoth bone;

three from the femur and three from the humerus (Waters and Stafford, 2014). Two of the dates were on XAD-purified collagen and are $12,300 \pm 35$ BP (UCIAMS-12308) for the femur and $12,270 \pm 35$ BP (UCIAMS-127316) for the humerus. The three XAD ages overlap at 1σ and average $12,300 \pm 25$ BP (Waters and Stafford, 2014). All of these radiocarbon measurements for the Lindsay Mammoth Site range from 9490 to 12,330 BP, with the youngest measurements being used to support the conclusion that mammoths survived into the Holocene and the older ages supporting evidence of pre-Clovis human presence (Krasinski, 2010; Waters and Stafford, 2014) (Table 9).

A bone from the same mammoth was selected for dating by the HYP method at the ORAU. Visual analysis of the sample revealed a coating of an unknown consolidant. Based on FTIR analyses we suspect that the contaminant was Butvar B-98. The collagen was therefore extracted after a solvent wash. The dating of hydroxyproline produced an age of $12,395 \pm 55$ BP (OxA-X-2736-15), which is congruent with all the XAD dates produced by Waters and Stafford (Huber and Hill, 2003; Waters and Stafford, 2014). It also overlaps at 2σ with the gelatin date of $12,290 \pm 35$. This new single-compound date reinforces the idea that this site predates the Clovis period by approximately 1,350 radiocarbon years. Despite the absence of classic lithic artifacts, taphonomic evidence from bone breakage and skeletal element distribution strongly implies human occurrence at the site.

Table 9: Previously published dates for the samples from Lindsay Wyoming, USA. Lab codes: S for Saskatchewan (Canada), Beta for Beta Analytic (USA) and WSU for Washington State University (USA).

Sample Number	Sample Preparation	^{14}C y BP (1σ)	References
I-7028	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$9,490 \pm 135$	(Davis and Wilson, 1985)
WSU-652	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$10,700 \pm 290$	(Davis and Wilson, 1985)
I-9220	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$10,980 \pm 225$	(Davis and Wilson, 1985)
Beta-102031	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$11,500 \pm 80$	(Davis and Wilson, 1985)
S-918	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$11,925 \pm 350$	(Davis and Wilson, 1985)
CAMS-82416	KOH extracted collagen (A+B+C+D2, Fig. 1)	$12,105 \pm 40$	(Huber and Hill, 2003)
CAMS-80541	Gelatin (A+B+C+D3, Fig. 1)	$12,175 \pm 40$	(Huber and Hill, 2003)
CAMS-72348	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	$12,330 \pm 50$	(Huber and Hill, 2003)
UCIAMS-127306	KOH extracted collagen (A+B+C+D2, Fig. 1)	$12,230 \pm 35$	(Waters and Stafford, 2014)
UCIAMS-127307	Gelatin (A+B+C+D2+D3, Fig. 1)	$12,290 \pm 35$	(Waters and Stafford, 2014)

UCIAMS-127308	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	12,300 ± 35	(Waters and Stafford, 2014)
UCIAMS-127309	KOH extracted collagen (A+B+C+D2, Fig. 1)	12,220 ± 35	(Waters and Stafford, 2014)
UCIAMS-127310	Gelatin (A+B+C+D3, Fig. 1)	12,255 ± 35	(Waters and Stafford, 2014)
UCIAMS-127316	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	12,270 ± 35	(Waters and Stafford, 2014)

5. Discussion

We obtained new ^{14}C dates for seven vertebrate fossils associated with four North American butchering sites: Wally's Beach (Canada), Dent (Colorado), La Prele, (Wyoming) and Lindsay (Montana). In 4 cases, we observed a discrepancy between the dates obtained on ultrafiltered collagen and the dates obtained on hydroxyproline. This shows that when samples are extremely contaminated, it can be difficult to totally remove the contamination from the collagen as it can also be chemically crosslinked. Similar observations have been made on a range of contaminated Palaeolithic bone samples dated at the ORAU (Becerra-Valdivia et al., 2018; Bourrillon et al., 2017; Deviese et al., 2017; Reynolds et al., 2017). Ages obtained on hydroxyproline are therefore to be preferred over those obtained from bulk collagen.

Radiocarbon ages obtained after the HYP and XAD procedures were compared using the Difference function in the OxCal 4.3 platform (Bronk Ramsey, 2018). The new ages obtained on hydroxyproline compare favorably with the previously reported XAD-derived ages for these sites with the exception of the *Mammuthus columbi* sample (SR-6606) from Dent, Colorado (Table 10). We also observe here that the ORAU dates tend to be slightly older. This is something currently being investigated, yet there are 2 likely explanations; (1) the dead carbon bleeding of the column is slightly underestimated for some of the samples (we monitor it regularly but it cannot be measured for every individual sample) or (2) the XAD is not removing a contaminant that is causing the collagen to date too young.

The new HYP ages from Wally's Beach (Canada) and Lindsay (Montana, USA) support the conclusion that these sites pre-date the Clovis time period, considering its start at 11,050 BP (Waters and Stafford, 2007). For the two Clovis sites, the HYP ages confirm the previous XAD purified protein hydrolysates from the Dent site and provide the first accurate age for the La Prele site: 13,041 to 12,757 Cal BP. This age falls well within range of the other fourteen well-dated Clovis sites from 13,050 to 12,650 Cal BP (Waters and Stafford, 2007).

415 Because contaminants were not completely removed during earlier dating efforts, the majority of XAD
416 and HYP ages are older than those previously obtained. However, a few of the dates derived on other
417 chemical fractions do come close. Sample of *Bootherium bombifrons* (Cat No. 3293.1), for example,
418 produced the same ages both on gelatin (UCIAMS-127372) and hydroxyproline (OxA-X-2736-9). This
419 is likely the result of either the absence of contaminants in the bone or the removal of all contaminants
420 during an earlier stage of pretreatment before purification using XAD and HYP methods (Fig. 1 and
421 Fig.2). In addition, as can be observed with samples from Dent, accuracy and precision of the ages
422 on chemical fractions from bone before XAD or HYP purification are inconsistent (Figure 3). It is
423 impossible to predict analytically, to a high degree of confidence, when methods other than XAD or
424 HYP are able to remove all of the contaminating carbon in dated bones. Sometimes they are reliable
425 and sometimes not. Only the XAD and HYP methods will consistently remove contaminants and yield
426 ages that are demonstrably contaminant free.

427

428 **Table 10:** Summary of HYP versus XAD ¹⁴C dates on fossil bones from North America.

SITE	Taxon	HYP Age ± 1σ RC yr.	AMS Lab No.	XAD Age ± 1σ RC yr.	AMS Lab No.	Age Difference, RC yr. (HYP - XAD)
Wally's beach, Canada	<i>Camelops</i> <i>hesternus</i>	11,530 ± 50	OxA-X-2736-8	11,465 ± 40 11,425 ± 30	UCIAMS-116400 UCIAMS-127347	-194 (95.4 %) 92 -237 (95.4 %) 21
	<i>Bootherium</i> <i>bombifrons</i>	11,255 ± 50	OxA-X-2736-9	11,320 ± 30	UCIAMS-127373	-88 (95.4 %) 171
	<i>Equus</i> <i>conversidens</i>	11,445 ± 55	OxA-X-2736-10	11,440 ± 30	UCIAMS-127351	-164 (95.4 %) 153
Dent, Colorado	<i>Mammuthus</i> <i>columbi</i>	11,055 ± 50	OxA-X-2736-11	10,960 ± 35	UCIAMS-116403	-290 (95.4 %) 70
	<i>Mammuthus</i> <i>columbi</i>	11,155 ± 50	OxA-X-2736-12			-355 (95.4 %) -36
La Prele, Wyoming	<i>Mammuthus</i> <i>columbi</i>	11,035 ± 50	OxA-X-2736-14	10,965 ± 30	UCIAMS-206764	-270 (95.4 %) 80
Lindsay,	<i>Mammuthus</i>	12,395 ± 55	OxA-X-2736-15	12,270 ± 35	UCIAMS-127316	-697 (95.4 %) 90

Montana	<i>columbi</i>					
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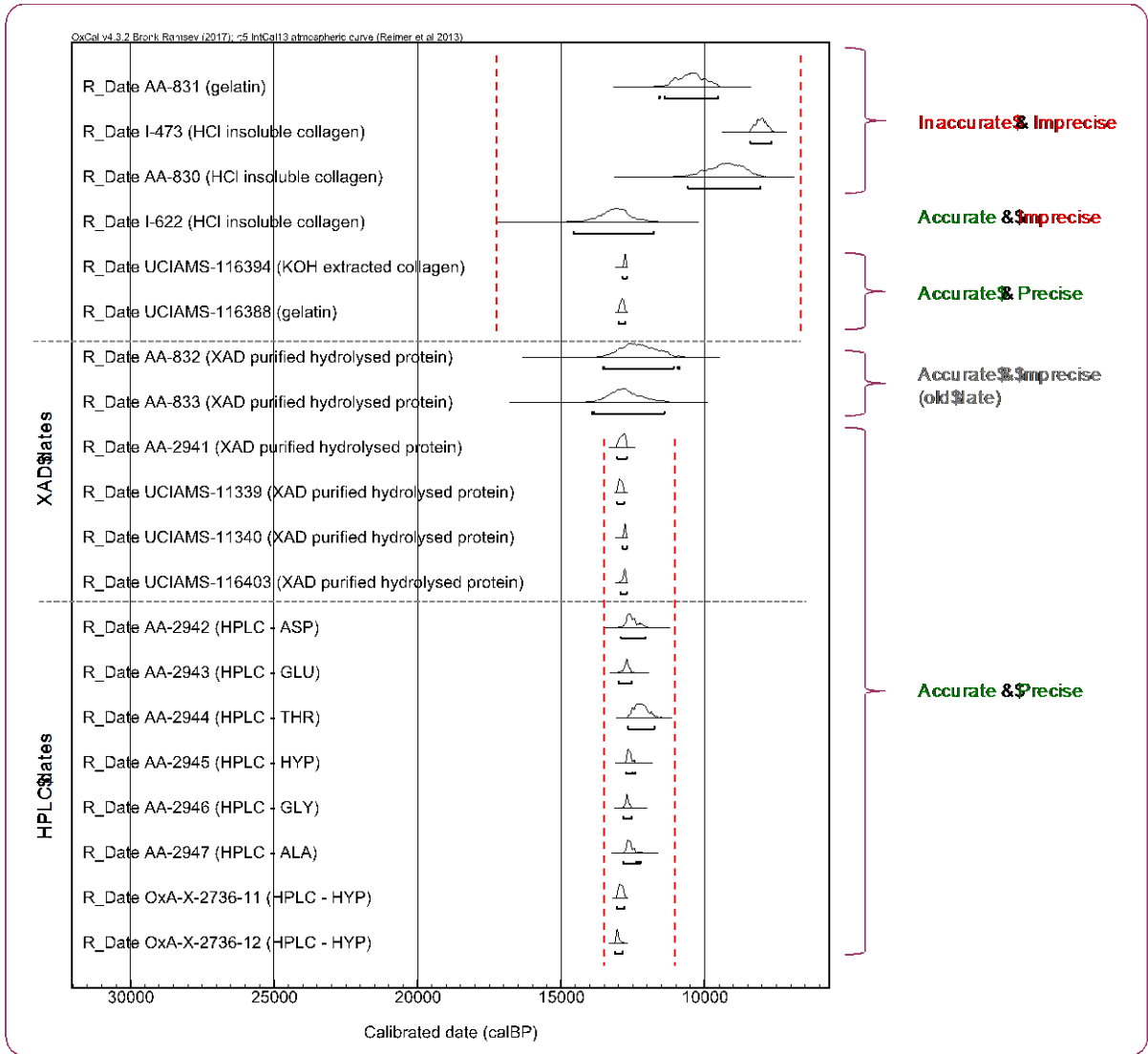


Figure 3: New calibrated hydroxyproline dates produced at ORAU for the samples from Dent, Colorado (USA), compared to previously-published measurements. This figure shows that most of the dates that have not been obtained using the XAD or HPLC approach, are too young and/or less precise.

Accurate dating of bones is necessary to properly reconstruct the story of the first Americans and Late Pleistocene megafaunal extinctions. Our study shows that XAD and HYP methods are more efficient in systematically removing contaminants from bone samples than other pretreatment methods and,

therefore, produce more reliable dates. We also demonstrated that when we examine determinations obtained using less robust methods, we encounter a range of results, some of which are reliable and some not. Ascertaining which samples are reliable is difficult as the analytical parameters used in assessing the degree to which the extracted collagen is intact and not subject to contamination are not always available. The C/N atomic ratio is, so far, the best quality indicator to identify if samples are heavily contaminated. It is acceptable for collagen (or protein hydrolysate isolated by XAD) if it ranges from 2.9-3.5. This indicator may however not be sensitive enough to detect contamination in small quantity or with a C/N ratio similar to the one of collagen. Hydroxyproline acts as a nearly unique biomarker for bone collagen and provides a chemically pure sample for accurate radiocarbon measurement. Measuring the C/N ratio of the hydroxyproline isolated using prep-HPLC is therefore the most sensitive and reliable indicator to check that the sample is totally free of contamination.

6. Conclusions

The debate concerning when and how humans first colonized the Americas is on-going and has been further intensified by analyses of aDNA from human remains. The accurate dating of human fossils is crucial to our understanding of this migration process. Due to the extreme rarity of Paleoindian human skeletons in the Americas, it is also important to directly date animal bones from kill and butchering sites. Our work has demonstrated how important sample purification chemistry is for obtaining accurate AMS ^{14}C measurements. More specifically, this dating effort on bones from Wally's Beach, La Prele (Fetterman), Lindsay, and Dent, illustrates that chromatographic methods, such as preparative High Performance Liquid Chromatography and column chromatography using XAD resins, are the most efficient methods in the removal of contaminants from bone samples. To avoid data misinterpretation, it is imperative that explanatory models in First Americans research only use radiocarbon determinations previously shown to be demonstrably accurate. This increased degree of accuracy is entirely dependent upon the most robust sample preparation chemistry that can be applied.

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