Geochemical Studies on Archived Samples from the Kennewick Skeleton: Research Plan for 2005 Laboratory Analyses

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Last Edited:
Monday, April 4, 2005
9:54 AM

On December 14 and 15, 2004, I examined the Kennewick Man skeleton and associated archived test samples. One of the purposes of my examination was to determine the most appropriate methods for making geochemical measurements on the skeleton and samples archived from previous radiocarbon and DNA analyses studies. The fossil bones are hard and will readily tolerate physical examination and measurement. The bone fragments and powders remaining from earlier (1999, 2000) government studies were more abundant, of larger mass and represented more different bones than I had expected. These archive samples are well suited for initial geochemical analyses. Using archived materials will reduce both the amount of time and the amount of intact bone that will be needed for geochemical testing of the skeleton.

I examined all archived samples and selected a subset of those that appear best suited for initial geochemical work. These samples are listed in Table 2. I am requesting that these bone fragments and bone powders be used for the chemical analyses described below. To the extent possible, I will save subsets of the archived samples for future comparisons and analyses.

Six different geochemical tests will be performed, but not all of them will be performed on each of the archived samples that I have selected. Some of the selected samples are too small for multiple tests. In addition, budget constraints limit the total number of tests that I can carry out at this time. Depending upon the results obtained from the initial tests, it may be necessary to perform some replicate tests to verify the initial test data. Most tests will consume approximately 20 to 50 milligrams of bone; isotope analyses will use 100 to 400 mg. I estimate that the tests proposed for this series of microsample investigations will collectively consume no more than five to eight grams of bone or bone powder.

The proposed tests and the reasons for conducting them are described in the following two sections. Other relevant details are provided in Tables 1, 2, 3 and 4.

Proposed Geochemical Tests on Archived Samples

1) **CHN and Quantitative Amino Acid Analyses** — estimate the amount of collagen present, its distribution within and among bones and the collagen's degree of chemical preservation.

2) **^{14}C Composition of Organic & Inorganic Phases** — AMS ^{14}C measurements on collagen and apatite will be indicators of the variability of $^{14}C/^{12}C$ ratios --- not ages --- in these organic and inorganic phases. The ^{14}C content varies depending upon bone diagenesis and contamination with foreign carbon. These data will be compiled to determine the best methods needed for redating the skeleton and for establishing why previous radiocarbon analyses vary by 2660 ^{14}C years.

3) **Elemental Analyses of Surface and Interior Elements**— electron microprobe analyses of external surfaces will determine how Fe and Mn, among other inorganic elements, are present as staining on those surfaces. Microprobe scans on polished cross sections will use organic nitrogen distributions to map collagen preservation within bones.

3) **SEM images and electron microprobe analyses of crystals filling bone & sediment voids** — the XYZ position and mineralogy of secondary crystal growths will help reconstruct orientation of the skeleton while it was in the ground and quantify diagenetic changes that were caused by percolating waters and biological agents.

4) **High resolution (50-100 µm) CT scans of bones** — these data are applicable to porosity differences in sediment infillings, location of secondary crystal growths, and especially the distribution of collagen within a single bone. Because the technique maps the density and distribution of collagen, the method is a non-destructive way to obtain proxy data concerning DNA content and will help detect if suitable regions remain where DNA could be extracted.

5) **Petrographic thin sections** — Standard petrographic thin sections examined under plain and polarized light and by cathode luminescence will give information on bone protein and bone mineral diagenesis, and secondary carbonate mineralization and foreign-crystal growth. Thin sections positioned along three planes at right angles will be combined with CT data to test these X-ray scans' reliability.

There are two reasons for the geochemical tests outlined above: 1) previous analyses were either incomplete or the results varied significantly among labs and 2) examination of the skeleton during December 14 to 15, 2004 suggested new taphonomic and geochemical data that could be obtained from the archived samples.

Prior analyses of the skeleton that are either incomplete or lacking precision include the following:

1) Quantitative amino acid analyses — only 16 of 18 possible amino acids were measured; data for secondary (imino) amino acids, proline and hydroxyproline, were not obtained and therefore exact collagen preservation was not determined.

2) Radiocarbon measurements vary by 2660 RC years on five specimens from the same skeleton —an age variation that is forty-four times measurement precision. Whether the skeleton is 8410 RC years or older is not established.

3) Stable carbon isotope (?^{13}C) values range from -10.3‰ to -21.9‰, a 11.6‰ range that is fifty times measurement precision. Neither an accurate paleodiet estimate or ^{14}C correction can be made from these ?^{13}C values.

4) Needed isotope measurements include ?^{15}N values, which would distinguish between terrestrial and marine-based diets, and ?^{18}O values that provide information that could help determine the geographic origin for the human.

5) All skeletal elements were not examined by non-destructive and non-invasive instrumental techniques that would assess the large inter- and intra-bone variations in collagen content and DNA potential.

8) Iron and Mn oxides remain unanalyzed to determine their origin.

New observations during the December 2004 inspection visit included the following, all of which can be quantified by analyses proposed in this summary:

1) The mineralogy and isotopic composition of calcium carbonate and carbonate-cemented sediments can be determined by using these archived bone fragments; no damage will occur to intact skeletal bone.

2) Sediments in the bone's medullary cavities contain vesicular structures and small (1 to 2 mm diameter) cavities filled with 10 to 50 μm long acicular crystals. Because both the vesicular structures and the secondary crystals occur in archived and intact skeletal bone, the origin and significance of sediment changes can be determined without altering intact bone.

3) Collagen distribution is very heterogeneous and can be mapped by using non-destructive CT scans. These biogeochemical assessments will determine what bones are most suitable for ^{14}C and DNA analyses.

The geochemical studies on archived bones will provide: 1) more precise and accurate data about the distribution and preservation of collagen, and therefore DNA, in all bones, 2) reasons why ^{14}C and stable isotope data vary, 3) additional taphonomic tools for determining orientation of the skeleton while it was in the ground, 4) empirically-based assessments of the skeleton's actual potential for yielding valid genetic and paleodietary information. Equally important, the amount of new intact bone that will be needed for future DNA and other tests will be dramatically reduced by having first performed quantitative tests on bone already removed from the skeleton.

TABLE 1

Summary of AMS radiocarbon measurements, stable isotope analyses and laboratory observations on the Kennewick Skeleton

Sample & ID No.	Age, RC yr. BP[1]	Apparent ^{14}C Age and Fm[2]	Lab Number	Fraction Dated	$\partial^{13}C$ ‰ PDB	Comments
5TH METACARPAL						
APS-CPS-01	8410±60	—	UCR-3476	Total amino acids	-15.4‰	Collagenous, well preserved bone; 68.8% of modern protein content
1ST RIGHT METATARSAL						
CENWW.97.R.24 (Mta) DOI-1a	8410±40	—	Beta-133993	Alkali-washed collagen	-12.6‰	"Plenty of Carbon"
CENWW.97.R.24 (Mta) DOI-1a	—	"8130±40" Fm = 0.3633±0.0014	UCR-3807	Total amino acids	-10.8‰	Non-collagenous protein content; 14% of modern
LEFT TIBIA (CNEMIAL CREST)						
CENWW.97.L.20b DOI2a	—	"5750±100" Fm = 0.4889±0.0066	AA-34818	Gelatin	-21.9‰	Below protein yield acceptable for accurate dating
CENWW.97.L.20b DOI2b	—	"6940±30" Fm = 0.4216±0.0015	UCR-3806	Total amino acids	-10.3‰	Non-collagen protein composition; 2.3% of modern
COMMENTS						
VARIATION AMONG RESULTS	**2660 ^{14}C yr. Range**			**"Collagen" to "Non-collagen"**	**11.6 ‰**	**"Acceptable" to "Not Acceptable" Results**

Notes: (1) Formal ^{14}C age. (2) An "apparent" ^{14}C age is a value not accepted by the AMS lab as a valid, absolute geological age for the sample. The Fraction Modern (Fm) is the sample's $^{14}C/^{12}C$ ratio but this value is not considered a geological age by the reporting lab.

TABLE 2

List of Archived Kennewick Man Samples

Suitable for Geochemical Analysis

2004KW-51

University of Arizona ^{14}C samples. Left Tibia fragments (97.L.20b) AA-34818b and AA-34818c. Archive vial No. 3.

2004KW-52

University of AZ ^{14}C samples. Left Tibia fragment (97.L.20b). Archive vial No. 4. AA-34818d. Doug Owsley relocated this fragment into its original position on the tibia.

2004KW-53

University of AZ ^{14}C samples. Left Tibia fragments (97.L.20b). Powder in scintillation vial.

2004KW-54

UC-Riverside Microsamples, A3-d. UCR-3876. Vial No. 2. Fine cortical fragments.

2004KW-55

UC-Riverside Microsamples, A3-d. UCR-3877. Vial No. 3. Small cortical fragments.

2004KW-56

UC-Riverside Microsamples, A3-d. UCR-3878. Vial No. 4. White cortical fragments.

2004KW-57

UC-Riverside Microsamples, A3-d. UCR-3881. Vial No. 7. Cancellous fragment.

2004KW-58

UC-Riverside Microsamples, A3-d. UCR-3882. Vial No. 8. Approximately ten cancellous & cortical fragments.

2004KW-59

UC-Riverside Radiocarbon Sample. UCR-3806. Left tibia 97.L.20b/DOI2b. Vial with white cortical bone fragments.

2004KW-60

Yale University Microsamples. Rib fragments from 97.L.12d(13), 50 ml plastic centrifuge tube with blue top.

2004KW-61

Yale University Microsamples. Metacarpal fragment 97.L.16(Mca) in 50 ml plastic centrifuge tube.

TABLE 2 (cont'd)

2004KW-62

Chatters original sample from University of Michigan Right (?) metacarpal sample 99.I.16b, 99.I.16c. [Possibly 99.L.16b and c ?]

2004KW-63

University of Michigan microsamples. Small (4-5 mm) diameter cortical bone fragment. Sample 97.L.16 (Mca).

2004KW-64

University of Michigan microsamples. Cancellous fragments, meta 2 and 3 [presumably meta*carpals* 2 & 3]. Sample 97.L.16 (Mca).

2004KW-65

Beta Analytic radiocarbon sample, A3-h. Aluminum foil packet with powder from Beta-133993. Right metatarsal sample 97.R.24 (Mta)

2004KW- number not assigned

University of California Riverside sample UCR-3807. Right Metatarsal 97.R.24(Mta)/00I1b, proximal end. [Comment — this sample was examined, but no 2004KW- number was assigned]

TABLE 3 Initial Tests to be Conducted on Each Selected Sample

CATALOG NUMBER	CHN	AMINO ACIDS	^{14}C	SEM ELEMENTS	SEM-CRYSTALS	CT SCAN	THIN SECTIONS	$?^{13}C$, ^{15}N,	^{18}O	SAMPLE ORIGIN
2004KW-51 L. Tibia frag (97.L.20b) AA-34818c	X	X	X			X	X	X		**UNIVERSITY of ARIZONA**
2004KW-52 L. Tibia frag. (97.L.20b) AA-34818d	X	X	X	X	X	X	X	X	X	
2004KW-53 Left Tibia frag. (97.L.20b)	X	X								
2004KW-54 UCR-3876	X	X						X		**UNIV. of CALIF. RIVERSIDE MICROSAMPLES**
2004KW-55 UCR-3877	X	X						X		
2004KW-56 UCR-3878	X	X						X		
2004KW-57 UCR-3881	X	X						X		
2004KW-58 UCR-3882	X	X						X		
2004KW-59 L. tibia frag 97.L.20b/DOI2b UCR-3806	X	X	X			X	X	X		**UCR - ^{14}C**
2004KW-60 Rib frags 97.L.12d(13)	X	X	X	X	X	X	X	X		**YALE UNIVERSITY**
2004KW-61 Metacarpal fragment 97.L.16(Mca)	X	X	X			X	X	X		

2004KW-62 metacarpal sample 99.I.16b, 99.I.16c	X	X	X					X		UC-DAVIS
2004KW-63 Sample 97.L.16 (Mca).	X	X				X		X		UNIV. of MICHIGAN
2004KW-64 97.L.16 (Mca).	X	X				X		X		
2004KW-65 R. metatarsal frag 97.R.24 (Mta) Beta-133933	X	X	X					X		BETA ANAL-YTIC
2004KW- NA UCR-3807, proximal end 97.R.24(Mta)/00I1b	X	X	X			X		X		UCR - ^{14}C

TABLE 4

Concordance Between Identification Numbers Used by USACE, Testing Facilities and Stafford on Samples Selected for Stafford Microsampling

Primary Container Label:
Kennewick Collection: Radiocarbon Samples from NSP [*sic*] Arizona AMS Facility A3-c

1. **Sample 2004KW-51**

Corps:	97.L.20b Vial with bone dust ("C") 15.8g (vial)
Arizona Vial label:	5 AA34818c
Stafford label:	Stafford μ Sample **2004KW-51** 15 Dec 2004

2. **Sample 2004KW-52**

Corps:	97.L.20b Vial with bone dust ("D") 16.2g (vial)
Arizona Vial Label:	4 AA34818d
Stafford Label:	Stafford μ Sample **2004KW-52** 15 Dec 2004

3. **Sample 2004KW-53**

Corps:	97.L.20b Vial with bone dust ("E") 15.5 g (vial)
Arizona Vial Label:	5 AA34818e
Stafford Label:	Stafford μ Sample **2004KW-53** 15 Dec 2004

Primary Container Label:
Kennewick Collection: Micro-Samples from UC Riverside
A3-d

4. **Sample 2004KW-54**

Corps:	97.I.12d(13) Vial with bone fragments (3876) within larger vial 11.7 g (inner vial)
UCR Vial Label:	3876 1.79 mg 2
Stafford Label:	Stafford μ Sample **2004KW-54** 15 December 2004

5. **Sample 2004KW-55**

Corps:	97.I.12d(13) Vial with bone fragments dust (3877) within larger vial 11.6 g
UCR Vial Label:	3877 194.6 mg 3
Stafford Label:	Stafford μ Sample **2004KW-55** 15 December 2004

6. **Sample 2004KW-56**

Corps:	97.R.16(MCa) Vial with bone fragments (3878) within larger vial 11.5 g (inner vial)
UCR Vial Label:	3878 0.96g 4
Stafford Label:	Stafford μ Sample **2004KW-56** 15 December 2004

7. Sample 2004KW-57

Corps:	97.L.16(MCb) Vial with bone fragments (3881) within larger vial 11.9g (inner vial)
UCR Vial Label:	3881 387.2g 7
Stafford Label:	Stafford μ Sample **2004KW-57** 15 Dec 2004

8. Sample 2004KW-58

Corps: 97.L.20b Vial with bone fragments (3882) within larger vial 14.0g (inner vial)

UCR Vial Label:	3882 2.48g 8
Stafford Label:	Stafford μ Sample **2004KW-58** 15 December 2004

Primary Container Label:
Kennewick Collection: Radiocarbon samples from UC Riverside
A3-b

9. Sample 2004KW-59

Corps:	97.L.20b vial with bone fragments (3806) 10.4g (vial)
UCR Vial/Bag Label:	Department of Interior, US Army Corps of Engineers, AMS Sample, Human Bone, Left Tibia shaft fragment (distal most), 5.3 grams, cat # CENWW.97.L.20b/DOI2b, Date: 8 Sept 99, Receipt: 10/26/00, Inventory Confirmed: 11/1/00, UCR 3808, 1.02g
Stafford Label:	Stafford μ Sample **2004KW-59** UCR-3806 15 Dec 2004

Primary Container Label:
Kennewick Collection: Micro-Samples from Yale University
A3e

10. Sample 2004KW-60

Corps:	97.I.12d(13) 50 ml vial (blue top) with 2 bone fragments 17.4 g (vial)
Yale University Label (vial):	97.1.12d(13) frag rib fragment Kennewick 6/10/00 FAK
Stafford Label:	Stafford μ Sample **2004KW-60** Rib 97.L.20b/DOI2b **15** Dec 2004

11. Sample 2004KW-61

Corps:	97.L.16MCa 50 ml vial (blue top) with bone fragments 15.2 g (vial)
Yale University Label(vial):	97.I.16d(MCa) metacarpal frag fragment 6/10/00 FAK
Stafford Label:	Stafford μ Sample **2004KW-61** Metacarpal 97.I.16 **(MCa)** 15 Dec 2004

Primary Container Label:
Kennewick Collection: DNA Samples from UC Davis CENNW.99.I.16
A3-g—See also Container A3-a

12. Sample 2004KW-62

Corps:	99.I.16b & 99.I.16c Ziploc bag of bone fragments 1.7g (bag)
UC Davis Label:	Original Chatters sample, 5th metacarpal right? MT 27 .129g
Stafford Label:	Stafford μ Sample **2004KW-62** 97.I.16b 97.I.16c 15 Dec 2004

Primary Container Label:
Kennewick Collection: Micro-samples from University of Michigan
A3-f

14. Sample 2004KW-63

Corps:	97.L.16(MCa) Ziploc bag with bone fragments 3.4 g (bag)
U Michigan Label:(bag):	University of Michigan, Sample 2-fragments after drilling. Receipt 10/30/00; Inv Conf 11/1/00. (inner bag): Kennewick metacarpal frag after drilling 5-23-00 DA?
Stafford Label:	Stafford μ Sample **2004KW-63** Metacarpal 15 Dec 2004

15. Sample 2004KW-64

Corps:	97.L.16(MCa) 15 ml vial with bone fragments 7.0g (vial)
U Michigan Label (bag):	University of Michigan, Sample 2-metacarpal fragments, Receipt 10/30/00 Inventory Confirmed 11/1/00. (vial): Kennewick 97.I.16 (Mca) left on from meta2 and meta 3…5-23-00 [illegible words]
Stafford Label:	Stafford μ Sample **2004KW-64** Meta 2 & 3 15 Dec 2004

Primary Container Label:
Kennewick Collection: Radiocarbon samples from Beta Analytic, Inc.
A3-h

16. Sample 2004KW-65

Corps: 97.R.24 (MTa)	2 ziploc bags with aluminum foil packet in which residue is contained (contents do not confirmed due to packaging)
Beta Analytic Label:	Label outside bag: (A3-h): 97.R.24 (MTa) 2 Ziploc bags with aluminum foil packet in which residue is contained (contents not confirmed due to packaging); on inner bag: 3.7g B-133993, McManamon DOI-1a, bone, unPit poc…qual
Stafford Label:	Stafford μ Samples **2004KW-65** Beta-133993 15 Dec 2004

Primary Container Label:
Kennewick Collection: Radiocarbon samples from UC Riverside
A3-b

17. Sample 2004KW-NA

Corps: 97.R.24(Mta)/00I1b	Proximal end. UCR-3807, Ziplock containing glass vial with mostly cancellous bone, but dense cortical bone that is very suitable for AAA and possibly ^{14}C dating.
Stafford Label:	None assigned