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Using bone bioapatite yield for quality control in stable isotope analysis applications

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ABSTRACT

Since the late 1970s, stable isotope analysis of bone has become a routine method in archaeological science for reconstructing an individual's life history. There is published guidance for assessing bone quality prior to stable isotope analysis, including measurement of bioapatite molecular structure, collagen yield, and collagen element content. This study investigated bioapatite yield as an additional quality indicator. Bioapatite and collagen were extracted from 182 modern (20th Century) and 272 prehistoric (5420–230 cal BP) human skeletal elements. As expected, modern remains were well-preserved, with ranges of 3–28% for collagen yield and 3.1–3.4 for the atomic carbon-to-nitrogen (C:N) ratio; bioapatite yield ranged from 21% to 63%. There was a significant, but only fair, negative correlation between bioapatite and collagen yields of modern samples ($r = -0.31$, $p < 0.0001$). Conversely, no correlation was observed between bioapatite and collagen yields of prehistoric samples, suggesting preservation of one is not indicative of preservation of the other. Prehistoric sample condition was evaluated by measuring the infrared splitting factor (IR-SF) to evaluate bioapatite crystallinity and the carbonate-to-phosphate (C/P) ratio to measure carbonate concentration; yield and C:N ratio were used to evaluate collagen condition. Prehistoric samples in the best condition had bioapatite yields between 29% and 62%, within the range of modern samples. In contrast, prehistoric samples in poor condition had significantly higher bioapatite yields, suggesting diagenetic addition of material. The range of bioapatite yields from modern bone samples, 21–63%, is proposed as the least conservative acceptable threshold for screening bone prepared for stable isotope analysis.

1. Introduction

Stable isotope analysis of human bone is useful for reconstructing an individual's life history because you are what you eat and drink, isotopically. Analysis typically focuses on: (1) the mineral phase, known as hydroxyapatite, or simply bioapatite; or (2) the organic phase that is commonly called "collagen" although it includes other proteins as well (Ambrose, 1990). Bioapatite is more abundant; the ash weight fraction in fresh human bone is approximately 58–66% (Kendall et al., 2018; Trotter and Hixon, 1974; Wang et al., 2001). In comparison, the weight fraction of collagen has been measured at 26–35% (Ambrose, 1990; Kendall et al., 2018; Wang et al., 2001), although 22% is frequently cited for fresh human bone (van Klinken, 1999).

The stable isotope analysis of both bioapatite and collagen can be useful for dietary reconstruction since bioapatite reflects the isotopic composition of the whole diet while collagen is biased toward the isotopic composition of consumed proteins (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). For reconstructing the isotopic composition of an individual's drinking water—to investigate geographic origin, migration, travel movement, etc.—the oxygen isotopes in bioapatite are typically measured (Daux et al., 2008; Levinson et al., 1987; Longinelli, 1984; Luz et al., 1984). Recent studies have also examined the relationship between the hydrogen isotopes in water and collagen (France et al., 2018; Reynard et al., 2020; Topalov et al., 2019), although other

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studies have documented a stronger relationship between the hydrogen isotopes in food and collagen (Gröcke et al., 2017; van der Sluis et al., 2019).

There are a variety of published methods on the preparation of bioapatite and collagen for stable isotope analysis. To extract bioapatite, bone is typically cleaned and powdered, then oxidized to remove the organic phase (Crowley and Wheatley, 2014; Koch et al., 1997). An additional treatment with (buffered) acetic acid is often used to remove the most labile carbonates, which are thought to be primarily diagenetic in origin (Garvie-Lok et al., 2004; Koch et al., 1997). To extract collagen, the simplest preparation methods use a weak acid to demineralize whole bone pieces (Sealy et al., 2014; Tsutaya et al., 2017). The resultant pseudomorph can be treated with a base to remove humic acids (Sealy et al., 2014) and/or solubilized in a process called "gelatinization" using heat and acid to homogenize the collagen (Longin, 1971). Gelatinization may be followed by filtration (Brown et al., 1988) before the collagen is freeze-dried.

To reconstruct life history using stable isotopes, the mineral and organic phases of bone need to retain similar isotope ratios as occurred in the living organism. However, the material compositions—and, by association, the isotopic compositions—of bioapatite and collagen can be impacted by diagenesis (Hoke et al., 2019; Nelson et al., 1986). Broadly defined as the degradation of bone due to biological, chemical, or physical factors, diagenesis is either an additive or subtractive process that may not be obvious at the macroscopic level. Some common factors include groundwater exposure, microorganism activity, soil pH, and temperature (Hedges, 2002). Often a concern when working with historic or prehistoric remains, even remains encountered in modern forensic casework could be affected by diagenesis – for example, through exposure (Saul, 2017), storage (Gordon et al., 2018), or chemical preservation (France et al., 2015, 2011).

Due to the potential impact of diagenesis, quality assessment techniques have been developed to evaluate the condition of both bioapatite and collagen in bone. Quality metrics for collagen are more established and traditionally include weight fraction (or, % yield), carbon content (wt%C), nitrogen content (wt%N), and atomic carbon-to-nitrogen (C:N) ratio (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). These collagen quality criteria are frequently used in radiocarbon dating applications [e.g., (Brock et al., 2012, 2007; van Klinken, 1999)]. In stable isotope analysis applications, all but collagen yield can be measured via elemental analysis (EA) during isotope ratio mass spectrometry (IRMS). For bioapatite, screening focuses on an examination of molecular structure; techniques used include histology, infrared spectroscopy, and x-ray diffraction (Hedges et al., 1995; Smith et al., 2007; Zazzo and Saliège, 2011). One commonly used screening tool is Fourier transform infrared (FTIR) spectroscopy, which characterizes two features of bioapatite: (1) crystallinity, as measured with an infrared splitting factor (IR-SF); and (2) the ratio of carbonate-to-phosphate ions (C/P). In contrast to its utility in stable isotope analysis applications (Beasley et al., 2014), the quality assessment of bioapatite molecular structure has proven to have limited utility for radiocarbon dating applications (Zazzo and Saliège, 2011).

As noted previously, it is possible to use collagen yield as a quality indicator for bone condition. However, to date there has been little guidance on the potential use of bioapatite yield for quality control. A 2006 study on Neolithic remains calculated bioapatite yields—and the related wt%C of carbonate—to evaluate diagenesis (Hu et al., 2006), but it does not appear that the practice has been widely adopted. This is surprising because, unlike IR-SF or C/P ratio, bioapatite yield requires no specialized instrumentation or training for measurement. It can be acquired prior to stable isotope analysis, potentially preventing the submission of "bad" samples for analysis. In addition, measurement of bioapatite yield could address needs related to quality control monitoring within accredited forensic programs, particularly by demonstrating repeatability and reproducibility of sample preparation methods.

In this study, we investigate the utility of bone bioapatite yield to screen samples prior to stable isotope analysis. As no thresholds exist for acceptable bioapatite yield, we first discuss limits using data collected from modern (20th Century) remains. We then assess their suitability using prehistoric human remains found to be in good condition following analysis via FTIR spectroscopy and EA-IRMS.

2. Materials and methods

2.1. Bone samples

A total of 182 skeletal elements from 20th Century remains, hereafter referred to as modern remains, were used to set thresholds of acceptable bioapatite yields. Thresholds were evaluated using 272 skeletal elements from prehistoric remains. Modern samples were taken from human long bones and were part of identification work at the Defense POW/MIA Accounting Agency (DPAA) Laboratory. Although the DPAA Laboratory regularly encounters preserved (e.g., chemically treated) remains from exhumations, only data from untreated remains were used in this study.

The prehistoric human remains were excavated from two archaeological sites in California, USA (CA-CCO-548, $n = 157$; and CA-SCL-038, $n = 115$) and date from approximately 5420–230 cal BP. Both sites have similar soil compositions, consisting primarily of alluvial deposits with silt, clay, sand, and gravel; as such, the remains from these sites may not be representative of the full range of diagenetic conditions worldwide. Samples from prehistoric remains were taken from long bones as well as ribs.

All samples (~1 g) were physically abraded using a rotary tool to remove surface contaminants. Bone pieces were then solvent cleaned to remove abrasion debris and lipids in sequential ultrasonic baths of deionized water, 95% ethanol, and 100% ethanol. These cleaning solvents do not preferentially remove either bioapatite or collagen. Finally, the samples were oven-dried (60–90 °C). Cleaned, dried bone was carefully weighed prior to extraction. Error on measurements was ± 0.001 g.

2.2. Bioapatite extraction

Bioapatite was extracted from modern bone at the Stable Isotope Preparation Laboratory at California State University, Chico following published guidance from Koch et al. (1997) and Crowley and Wheatley (2014). Briefly, a piece of cleaned bone was powdered using a steel mortar and pestle and sieved (200 μm mesh). The powder was weighed and then treated with 30% hydrogen peroxide (H_2O_2) at a volume of 0.04 ml solvent to 1 mg sample for 48 h at room temperature; the hydrogen peroxide was changed once, at 24 h. Samples were next treated with 1.0 M acetic acid (CH_3COOH), buffered to pH 4.5 with sodium hydroxide (NaOH), at a volume of 0.04 ml solvent to 1 mg sample for 24 h at room temperature. The acid solution was changed once, at 12 h. The resultant bioapatite was oven-dried, and its mass was compared to the mass of the original, powdered bone sample to calculate bioapatite yield, expressed in weight percentage with an uncertainty of 0.1% based on balance error.

Bioapatite was extracted from prehistoric bone in the same manner, with one notable variation in the oxidation process: a 1.5% solution of sodium hypochlorite (NaOCl) was used in lieu of hydrogen peroxide. A representative selection of bioapatite extracted from prehistoric remains was examined microscopically for the presence of sediments, such as silts or clays; these could erroneously contribute to yield by affecting bioapatite mass. Embedded sediments were found to represent < 5% per unit volume in the prehistoric bioapatite samples, indicating no meaningful contribution to the overall yield.

2.3. Collagen extraction

Collagen was extracted using the "chunk" method published by Sealy et al. (2014), with an additional gelatinization step. Briefly, cleaned bone pieces were completely demineralized in 0.10–0.25 M hydrochloric acid (HCl) at room temperature for approximately 15–30 days. The demineralized bone was next treated with 0.125 M NaOH for 24 h. The resultant collagen pseudomorph was solubilized using pH 3 water and heat (60–90 °C) over several days (Bartelink et al., 2020; Beasley et al., 2013). Collagen was freeze-dried and its mass was compared to the mass of the original bone piece to calculate collagen yield, expressed in weight percentage with an uncertainty of 0.1% based on balance error.

2.4. Bioapatite analysis

While the modern remains were assumed to be in good condition, it was likely that diagenesis had affected the prehistoric samples and their condition was therefore assessed using FTIR spectroscopy. Samples were prepared for analysis by grinding 1.5 mg extracted (treated) bioapatite with 200 mg potassium bromide (KBr) in an agate mortar and pestle. The mixture was pressed into a 3-mm disc in a hydraulic press at ~10,000 psi for 2 min to form a pellet. Pellets were analyzed at California State University, Chico in the Department of Chemistry on a Nicolet Magna 500 FTIR analyzer from 4000 to 400 cm^{-1} using 100 scans at a resolution of 8 cm^{-1}. The spectra were analyzed using OMNIC v7.0. Following Beasley et al. (2014), we used Weiner and Bar-Yosef's (1990) method for measuring IR-SF and Wright and Schwarcz's (1996) method for measuring the C/P ratio.

2.5. Collagen analysis

All collagen samples were analyzed via EA-IRMS at the Stable Isotope Facility at the Univeristy of California, Davis. The elemental concentration data collected during analysis [e.g., total N (μg), total C (μg), and the atomic C:N ratio derived from these] was used to assess sample condition. Only data related to material quality were used in this study and thus no isotope delta values are presented here, although the isotope test results for the prehistoric samples are available elsewhere (Bartelink, 2009, 2006; Bartelink et al., 2020; Gardner, 2013).

2.6. Statistics

Statistical analyses were performed using Prism 6 for Mac OS X. Prehistoric samples were grouped by condition for some statistical comparisons by assigning each sample a conditional score. The quality indicators used for scoring were collagen yield, C:N ratio, IR-SF, and C/P ratio. Acceptable ranges for indicators were as follows.

- Collagen yield: 3–28% [based on data from (Ambrose, 1990; Brock et al., 2007)]
- C:N ratio: 3.1–3.5 [from (van Klinken, 1999)]
- IR-SF: 3.00–3.60 [from modern bone measured by (Beasley et al., 2014); supported by measurements made on modern bone by (Dal Sasso et al., 2018; Lebon et al., 2010)]
- C/P ratio: 0.09–0.31 [from modern bone measured by (Beasley et al., 2014)]

Out of a total of four (4) points possible per sample, one (1) point was subtracted for each quality indicator outside the acceptable range. This generated a score that could range from 0 to 4, with 0 representing a sample in very poor condition and 4 representing a sample in the best condition. For example, a prehistoric sample with a collagen yield of 2% (unacceptable), a C:N ratio of 3.3 (acceptable), an IR-SF of 4.29 (unacceptable), and a C/P ratio of 0.09 (acceptable) would be assigned a total score of 2.

3. Results and discussion

Descriptive statistics of bone bioapatite and collagen extracts are provided in Table 1. Individual sample results are available in Appendix A (Supplementary data). We note that the mass of powdered bone was incorrectly recorded for one modern sample and it was impossible to calculate bioapatite yield as a consequence.

As expected, the modern samples were in good condition based on their collagen yields (%Coll) and C:N ratios, with ranges of 3–28% and 3.1–3.4, respectively. Bioapatite yields (%Ap) for modern samples ranged from 21% to 63%, with a mean of 42%. The mean bioapatite yield for prehistoric samples was significantly higher, at 50% (unpaired *t*-test, $p < 0.0001$). The mean collagen yield for prehistoric samples was significantly lower, at 10%, than the mean collagen yield of 18% observed for modern samples ($p < 0.0001$).

Correlations between bioapatite yield and the other paired sample quality control metrics were evaluated using the Spearman's rho (Akoglu, 2018). A significant, but only fair, negative correlation was observed between bioapatite yield and collagen yield for the modern samples ($r = -0.31$, $p < 0.0001$); as bioapatite yield increased, collagen yield tended to decrease. In contrast, there was no correlation between bioapatite yield and collagen yield for the prehistoric samples. This may suggest that the materials progressed through different diagenetic trajectories and the preservation of one—as assessed using yield data—is not indicative of the preservation of the other within bone. A fair positive correlation was observed between prehistoric sample bioapatite yield and IR-SF ($r = 0.43$, $p < 0.0001$), suggesting a relationship between bioapatite yield and increased sample crystallinity. In addition, the fair negative correlation between sample bioapatite yield and C/P ratio ($r = -0.40$, $p < 0.0001$) suggests high yields are associated with a loss of carbonate. As the C/P ratio measures carbonate in the phosphate position of the bioapatite lattice, this is indicative of a loss of structural carbonate and not diagenetic (labile) carbonate. Bioapatite yield was poorly correlated with C:N ratio for prehistoric samples ($r = 0.17$, $p < 0.01$).

The paired yields of bioapatite and collagen were summed for each sample; the totals ranged from 32% to 86% for modern samples and from 35% to 90% for prehistoric samples (see Appendix A). Some were well below the theoretical totals of bioapatite and collagen weight fractions in bone, which can be calculated from the literature and are approximately 80–100% (Ambrose, 1990; Kendall et al., 2018; Trotter and Hixon, 1974; van Klinken, 1999; Wang et al., 2001). We note that the theoretical totals are truly theoretical, as little data has been published on bioapatite yield actually observed during sample preparation in the laboratory (Hu et al., 2006). Inadvertent material loss during the various treatment steps and washes may be one cause of the lower-than-theoretical totals observed for both modern and prehistoric remains. Although the range of summed yields appears slightly lower for modern samples, there was no significant difference in the group means (unpaired *t*-test, $p = 0.2670$; mean = 60% for both). It appears the lower bioapatite yields of the modern samples were essentially offset by their higher collagen yields so that the combined material yields of the modern samples were similar to that of the prehistoric samples that had higher bioapatite yields but lower collagen yields.

Table 1
Descriptive statistics for bioapatite and collagen extracted from all modern and prehistoric bone samples.

	Modern samples			Prehistoric samples				
	%Ap	%Coll	C:N	%Ap	%Coll	C:N	IR-SF	C/P
N	181	182	182	272	272	272	272	272
Mean	42%	18%	3.2	50%	10%	3.3	3.62	0.16
SD	8%	7%	0.1	8%	8%	0.4	0.29	0.04
Min	21%	3%	3.1	29%	0%	3.2	2.94	0.04
Max	63%	28%	3.4	68%	34%	8.9	4.83	0.35

The methods used to extract bioapatite from modern and prehistoric bone may have had a differential impact on yields. Modern samples were treated with 30% hydrogen peroxide while prehistoric samples were treated with 1.5% sodium hypochlorite. Snoeck and Pellegrini (2015) found that hydrogen peroxide was not sufficient to remove all organic material from bone while sodium hypochlorite was an effective solvent for oxidation. Incomplete removal of "collagen" could result in a higher sample mass post-treatment, and artificially inflate bioapatite yield; however, in this study modern samples had on average lower yields than prehistoric samples. Instead, the acidic hydrogen peroxide solvent may have caused loss of bioapatite due to dissolution (Snoeck and Pellegrini, 2015), leading to lower bioapatite yields for modern samples. Conversely, the higher bioapatite yields for prehistoric samples may be due to carbonate precipitated during treatment with sodium hypochlorite (Crowley and Wheatley, 2014; Snoeck and Pellegrini, 2015). An additional acetic acid treatment was used to remove these precipitated carbonates (Garvie-Lok et al., 2004; Koch et al., 1997), but there is no guarantee the treatment was completely successful.

Fig. 1 presents just bioapatite yields for modern and prehistoric samples in a box plot; prehistoric samples have been separated into three groups based on condition. Bioapatite yield statistics for the prehistoric sample groups are provided in Table 2. In general, it appears bioapatite yield increased as sample condition degraded. Prehistoric samples in the best condition, grouped as Score 4, had on average significantly lower bioapatite yields than prehistoric samples in the worst condition, grouped as Scores 0–2 (one-way ANOVA with Tukey's multiple comparison, $p < 0.0001$). The samples grouped as Score 4 had on average significantly higher bioapatite yields than the modern samples (unpaired *t*-test, $p < 0.0001$; mean = 48% and 42%, respectively) – although, as previously discussed, this may be in part due to the different solvents used for oxidizing organic material in the prehistoric and modern samples.

Table 2
Bioapatite yield statistics for prehistoric bone samples, separated into three groups based on condition from worst (Score 0–2) to best (Score 4).

	Score 0–2	Score 3	Score 4
N	37	110	125
Mean	56%	52%	48%
SD	6%	7%	7%
Min	43%	33%	29%
Max	68%	65%	62%

As collagen and bioapatite preservation in the prehistoric samples are not necessarily linked, bioapatite yields were also compared when samples were separated into groups based solely on bioapatite quality indicators (i.e., IR-SF and C/P ratios; see Appendix A). Only five of 272 samples had unacceptable scores for both. One hundred twenty-six prehistoric samples had one acceptable bioapatite quality indicator while 141 had acceptable scores for both indicators. Prehistoric samples with acceptable scores for IR-SF and C/P ratio had on average significantly lower bioapatite yields than samples with only one acceptable score (unpaired *t*-test, $p < 0.0001$; mean = 48% and 53%, respectively). Once again, the data suggest bioapatite yield increased as sample condition degraded.

The distributions of bioapatite yields shown in Fig. 1 were more closely examined using histograms, shown in Fig. 2. Bioapatite yields for modern samples were normally distributed (Shapiro-Wilk normality test, $p = 0.919$) while those for the best condition prehistoric samples were not (Score 4; $p = 0.0089$). The distribution for Score 4 appeared bimodal, with one peak at 38–40% and another at 52–54%. The first, lower peak observed in the Score 4 distribution is similar to the peak about 38–42% observed for the modern samples; it is also similar to the modern group's mean bioapatite yield of 42% (see Table 1). The second, higher peak observed in the Score 4 distribution may indicate the

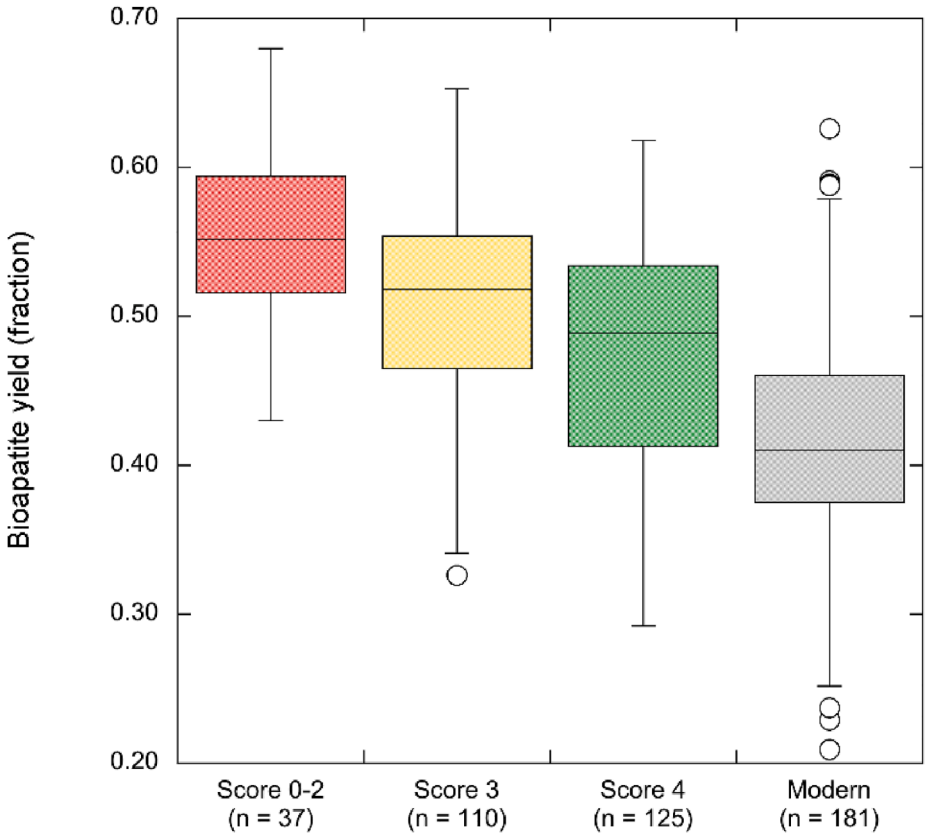

Fig. 1. Box plot of bioapatite yields from prehistoric bone samples, separated into three groups from worst (Score 0–2) to best (Score 4) condition, and from modern bone samples.

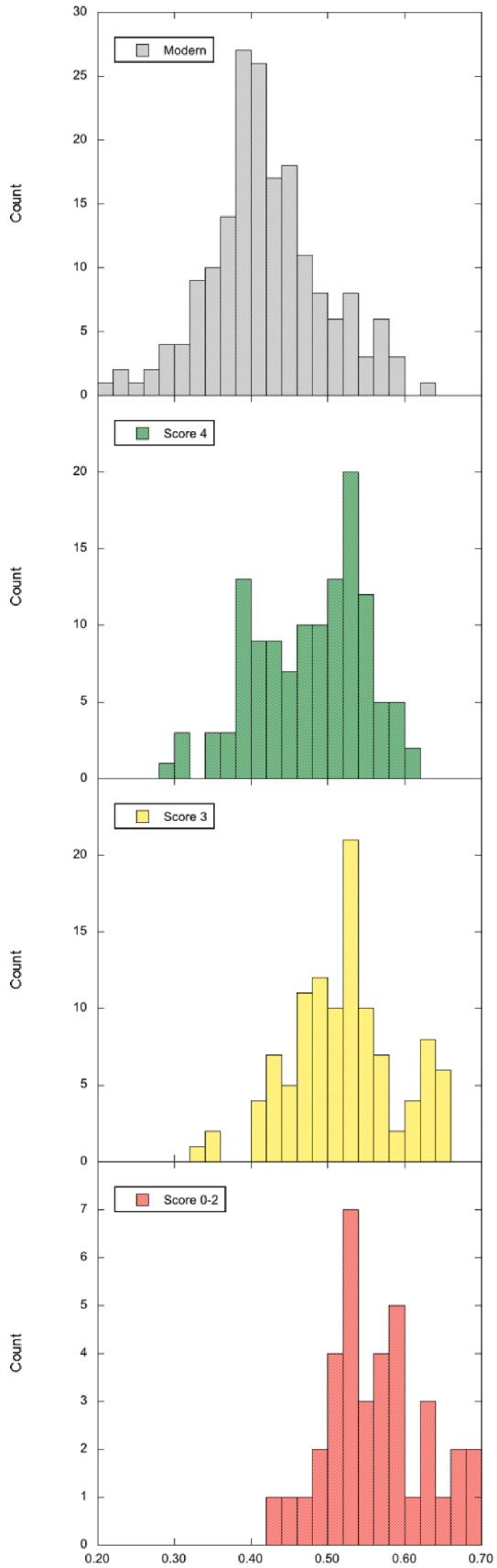

Fig. 2. Histograms of bioapatite yields of modern samples (top) and prehistoric samples, sorted into three groups from best (Score 4) to worst (Score 0–2) condition.

presence of diagenetic material in even the best condition prehistoric samples, which was not removed by the acetic acid treatment. This suggests that the preparation methods used in this study were not sufficient to completely eliminate all labile carbonate in the prehistoric remains.

As additional evidence of diagenetic material in the bioapatite extracted from prehistoric bone, the peak for Score 3 was at 52–54%, the same as the second peak observed for Score 4. The distribution was not normal (Shapiro-Wilk normality test, $p = 0.0374$), but there was no obvious bimodality in the bioapatite yields of this group. In contrast, the bioapatite yields for the worst condition prehistoric samples were normally distributed ($p = 0.1066$; see bottom panel, Fig. 2) although we note that the sample size for the group was small ($n = 37$). The peak for bioapatite yields of Score 0–2 samples was again at 52–54%. This also supports the hypothesis that diagenetic material was incorporated into all the prehistoric samples.

Despite the bimodality in the bioapatite yields of the best condition prehistoric samples (29–62%; see Table 2) and the difference in solvents used for oxidizing organic material, all yields for samples grouped as Score 4 fell within the range of modern samples (see Fig. 1 and 2). Bioapatite yields of an additional 74 modern bone samples recently prepared as part of the new isotope testing program at the DPAA Laboratory in Hawaii were 34–63% (see Appendix A) and also fell within the range of modern samples previously prepared at California State University, Chico. We thus propose that the absolute lower and upper thresholds for acceptable bioapatite yield should be set as 21% and 63%, respectively. The analysis of samples with bioapatite yields falling outside this range is not advised for forensic work, due to the potential effect of diagenesis on material composition – and, potentially, isotopic composition.

The absolute thresholds set using the minimum and maximum bioapatite yields of modern samples are not particularly conservative; as a consequence, 32 of 37 prehistoric samples grouped as Score 0–2 had "acceptable" bioapatite yields (see Appendix A). To exclude all prehistoric samples with Score 0, an upper threshold of 56% would be required. A 21–56% limit would exclude eight of 181 modern samples, for an exclusion rate of ~4%. To exclude all prehistoric samples with either Score 0 or 1, an upper threshold of 51% would be required and 21 modern samples would be excluded (~12%).

We recommend the use of bone bioapatite yield as a screening tool for follow-on stable isotope analysis, as it indicates the addition or loss of material due to diagenesis. In this study, a bioapatite yield range of 21–63% is endorsed as the benchmark for evaluating sample quality. It is the least conservative of the ranges suggested above. Reducing the range to 21–56% or even 21–51% will exclude most—but not all—poor condition samples (e.g., Scores 0 and 1), but will exclude some "good" condition samples as well.

Finally, we note that it may be possible to analyze the collagen phase of bone with unacceptable bioapatite yield. The data on prehistoric remains collected in this study suggested that bioapatite and collagen can progress through separate diagenetic trajectories. However, in this scenario, the use of other quality indicators for screening bone, such as collagen yield and element content should be employed (Ambrose, 1990; van Klinken, 1999).

4. Conclusions

This study investigated the utility of bioapatite yield for evaluating bone sample quality prior to stable isotope analysis. Using data collected from 255 total modern bone samples, we propose thresholds for acceptable bone bioapatite yield as 21–63%. These thresholds should be used to assess the quality of bone prepared for follow-on stable isotope analysis. Since preservation of bioapatite may not be indicative of the preservation of collagen, and vice versa, both phases should be monitored for quality. Prior studies have published guidance on using collagen yield, element content (Ambrose, 1990; DeNiro, 1985; van

Klinken, 1999), and bioapatite molecular structure (Beasley et al., 2014) for screening bone. We note that previous work focused primarily on prehistoric bone while the current study demonstrates the potential utility of bioapatite yield as an additional sample quality indicator specifically for modern remains.

Most of the aforementioned quality indicators for bone bioapatite and collagen require the use of specialized measurement techniques – i.e., FTIR spectroscopy or EA-IRMS. However, despite a considerable increase in the applications of stable isotope analysis to archaeology since the 1970s (Roberts et al., 2018; Szpak et al., 2017), many users do not have ready access to, or training on, the analytical instrumentation required for measurement. Users instead rely on external service providers for sample analysis – and, in some cases, sample preparation as well. In these instances, users must submit samples and then wait until analysis is complete to assess quality, potentially submitting (and paying for) poor condition samples. Calculating % yield following bioapatite extraction can provide researchers a useful sample screening tool prior to stable isotope analysis.

Whatever the method(s) employed to screen bone used in isotopic investigations, it is vitally important that sample quality is assessed. Any diagenetic process that affects material composition could lead to a "non-authentic" isotopic signature (Hoke et al., 2019) that does not accurately reflect the history of the living organism. The analysis of poor-quality samples—and the "bad" data such an analysis generates—can impact interpretations about an individual's past diet and drinking water [see, for example, discussions about origin prediction accuracies based on human hair data (Gordon et al., 2018; Saul, 2017)]. In archaeological settings, this may simply mean the incorrect identification of travelers within a prehistoric population (Lightfoot and O'Connell, 2016). However, the implications of misinterpretations in forensic applications of isotope analysis could be particularly serious if particular travel scenarios or geographic sources are excluded from consideration that should not be.

CRediT authorship contribution statement

Lesley A. Chesson: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Melanie M. Beasley:** Methodology, Investigation, Resources, Data curation. **Eric J. Bartelink:** Methodology, Investigation, Resources. **Miranda M.E. Jans:** Conceptualization, Methodology, Resources. **Gregory E. Berg:** Conceptualization, Methodology, Formal analysis, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2020.102749.

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