THE $\delta^{18}\text{O}$ OF PHOSPHATE OF ANCIENT HUMAN BIOGENIC APATITE CAN REALLY BE USED FOR QUANTITATIVE PALAEOCLIMATE RECONSTRUCTION?

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Abstract

It is well known that the oxygen isotope composition of phosphate apatite ($\delta_{\text{ph}}$) in mammal bones and teeth is strongly correlated to that of the drinking water ($\delta_{\text{w}}$) ingested by the mammal itself. However, the relation between ($\delta_{\text{ph}}$) and ($\delta_{\text{w}}$) has to be considered with caution when used for palaeoclimate reconstruction because of the uncertainty of the data. Usually, however, the authors underestimate this problem, that may led to misleading results. On the basis of the phosphate-water data existing in the literature for humans, we estimated that the prediction uncertainty for $\delta_{\text{w}}$ calculated from a new value of $\delta_{\text{ph}}$ is on the order of 2.5‰. It means that only in the case the difference between two calculated $\delta_{\text{w}}$ values is higher than about 3-4‰, the $\delta_{\text{w}}$ values may be considered significantly different. This represents a big limit which cannot be underestimated in the use of human $\delta_{\text{ph}}$ for prediction of single $\delta_{\text{w}}$ values and subsequent estimation of palaeotemperature. A similar evaluation would be performed also for other mammals.

Keywords: Oxygen isotopes, bioapatite, environmental water, prediction uncertainty, climate reconstruction

Introduction

Chemists always use error distribution laws to compute prediction uncertainty on data obtained by linear or more complex calibration. On the contrary, prediction uncertainty is not commonly estimated in stable isotopic studies of palaeoclimatology and physical archaeology. Thus, in this paper, we present a case of prediction uncertainty calculation applied to oxygen isotope distribution between the phosphate group of human bone bioapatite.
and the presumed drinking water. We demonstrate that this distribution must be used with great caution in archaeology and palaeoclimatic reconstruction.

The potential use of oxygen isotope measurements of mammal bone and tooth phosphate anion of bioapatite for palaeoclimatic research was demonstrated more than thirty years ago (Longinelli, 1974, 1984; Luz et al., 1984; Luz and Kolodny, 1985). Owing to the very slow biological replacement of the bone apatite, thermodynamic equilibrium between the phosphate anion and the body water may be supposed a priori. Bone and enamel phosphate (ph) of the biogenic apatite would acquire an isotopic composition$^4$, $\delta_{ph} (\equiv \delta^{18}O_{ph})$, which is independent from the environmental temperature and that, for a given species, gives constant ratio $(\delta_{ph}+1)/(\delta_{bw}+1) = \alpha_{ph/bw}$, where $\alpha_{ph/bw}$ is the fractionation factor between the bioapatite phosphate group, ph, and the body water, bw, and $\delta_{bw}$ is the delta value for the body water. For instance, for domestic pigs (Longinelli, 1984), the relationship between $(\delta_{ph}+1)$ and $(\delta_{bw}+1)$ is well represented by a regression line ($R^2 = 0.998$) whose intercept is not significantly different from zero. Thus, the obtained average ratio $(\delta_{ph}+1)/(\delta_{bw}+1) = 1.02082$ (4 couples of data) may be regarded as a good estimation of the oxygen fractionation factor, $\alpha_{ph/bw}$.

The oxygen input in human body is related to (1) drinking water, (2) ingested liquid water from food, (3) food (metabolic oxygen), and (4) atmosphere (oxygen uptaken in the lungs), whereas the output to (1) carbon dioxide production, (2) water vapour emission and (3) expulsion of urine, sweet, etc. Since the body water has variable provenance, the relationships between ingested water and the oxygen of the bone and tooth phosphate group is difficult to define. Luz et al. (1984) and Luz and Kolodny (1989) defined for the first time a model which took into account the input-output flux of oxygen in the animal body and the role of environmental humidity. The model was improved by Bryant and Froelich (1995) and Kohn (1996), who discussed the argument on biological basis, and, recently, reconsidered by Daux et al. (2008) and Podlesak et al (2008). Among the important variables of the model, such as the diet, the mass of the body, and the ingested water, the latter is crucial. These models account for the attempt of several researchers to derive empirical linear equations with the aim of relating $\delta_{ph}$ and $\delta_{w}$ of the drinking water for different animal species.

$^4$ According to the IUPAC (International Union of Pure and Applied Chemistry) recommendation, we consider $\delta = (R/R_{SMOW}) - 1$, where R and $R_{SMOW}$ are the isotope ratio in the sample of interest and in the international standard V-SMOW, respectively. Moreover, in defining the regression line, we use $(\delta+1)$ values in place of $\delta$ because it makes easier to recognise possible fractionation factors. $\alpha$. Symbols frequently used are reported in Table 1.
A very important questionable point is the following: from $\delta_{ph}$ of ancient or fossil enamel and bone is it really possible to make enough accurate estimation of the delta value of the palaeoenvironmental water and hence of palaeoenvironmental temperature?

This paper takes into consideration the published data regarding the measured $\delta_{ph}$ for humans (Longinelli, 1984; Luz et al., 1984; Levinson et al., 1987; Daux et al., 2008). The aim is to demonstrate by statistical computation that, although the oxygen isotope characteristics of presumed drinking water ($\delta_w$) and phosphate group of bone and tooth apatite ($\delta_{ph}$) are significantly correlated, the $\delta_{ph}$ values may be used with great caution for accurate prevision of the environmental water and, thus, for accurate inference of palaeotemperature.

**Evaluation of the data**

**What drinking water?**

The models cited before take into account total ingested liquid water. However, the authors who investigated oxygen isotopes in bones (Longinelli, 1984; Luz et al., 1984; Levinson et al., 1987; Daux et al., 2008) do not take into account the total ingested liquid water, but considered a presumed drinking water ($\delta_w$), i.e. precipitation water, modelled precipitation water and, in one case, tap water. Longinelli (1984) use meteoric water, which "were measured directly or taken from previous papers, from the data reported by IAEA, or were “extrapolated” from values obtained from nearby areas” (p. 386). This author does not indicate if the water data are averages on many years or just on one year and how the “extrapolation” (probably used in place of interpolation) of the meteoric water values was done. Levinson et al (1984) analysed directly drinking water from all the localities of interest; these authors, however, state that, since their isotopic values “come from a single sample measurement, they do not represent the entire range of water compositions which may be found in a specific locality” (p. 369). At last, Daux et al. (2008) report two sets of water data: values of measured tap waters as well as values estimated from a global dataset (International Energy Association/World Meteorological Organization) using an algorithm developed by Bowen and Wilkinson (2002) and refined by Bowen and Revenaugh (2003).

Assimilation of oxygen into the phosphate group of biogenic apatite is a slow process because the residence time of oxygen in phosphate is of the order of five to twenty years depending on the bone considered. Thus, any investigation on the relationship between the environmental water and phosphate should consider time-integrated values of the water. From the papers, however, it is not always evident if the author/s use long-term
integrated data or not; actually the time of integration would be comparable
to the residence time of oxygen in the phosphate of bioapatite.

In the opinion of Longinelli (1984), the value for meteoric waters
would be reliable within plus or minus a few tenths of one per mil. Luz et al
(1984) suggest a meteoric water uncertainty of 1‰ (1σ) at low latitudes and
2‰ at high latitudes. Daux et al (2008) do not give any indication of the
water values uncertainty. Thus the experimental standard deviation, s(δw), of
δw is not known with good approximation.

Summarising, we must remember that (a) meteoric waters are only
presumed drinking waters, (b) drinking and totally ingested water are not
effectively the same, (c) the assimilated oxygen does not come only from
ingested water, (d) uncertainty on the environmental water is not well known
and evaluated in different way by the different authors.

Phosphate δ18O of biogenic apatite

We have considered four published data sets from Longinelli (1984),
Luz et al. (1984), Levinson et al. (1987) and Daux et al. (2008). Several data
reported by Longinelli (1984) and by Luz et al. (1984) are averages of
different measurements (up to ten); thus, hereafter, in most cases, in place of
single measurements, we are compelled to use δph data which actually are
mean values of several measurements. Moreover, the reported experimental
standard deviation, s(ph), of the phosphate data is variable. Longinelli (1984)
states generically that the standard deviation of his δph measurements is
about 0.2‰; Luz et al. (1984) report a value of 0.5‰ as analytical
reproducibility; Levinson et al. (1987) and Daux et al. (2008) a value of
0.2‰. These analytical uncertainties, however, are of scarce relevance for
the investigation of the δph vs δw because the authors frequently report only
data which are averages of several measurements rather than the single
measurements.

The experimental standard deviation for the δph data reported by
Longinelli (1984) ranges from 0.15‰ to 0.5‰, for Luz et al. (1984) from
0.2‰ to 0.8‰ (data deduced from their Fig.1), for Levinson et al. (1987)
from 0.2‰ to 1.15‰, for Daux et al. (2008) from 0.2‰ to 0.8‰. The
distribution of these dispersions is not normal; thus, we used their median
(0.40‰) to define the central value, and the 16th percentile and 84th
percentile to estimate the dispersion (α = 0.32) around the true median M:
(0.40 - 0.20)%o ≤ M ≤ (0.40 + 0.39)%o. For our laboratory experience, the
dispersion of 0.40% may be considered as a satisfactory approximation of
measure reproducibility. In any case, for the purposes of our calculations,
this value is not really important as we will see later (Paragraph 4).
Aproximate uncertainty computation

Symbols used are reported in Table 1; the few reported bibliographic references are sufficient to understand the discussion.

To avoid ambiguity, the following considerations must be taken into account before approaching in detail the estimation of the uncertainties on \( \delta_w \). As discussed above, \( s(\delta_w) \) and \( s(\delta_{ph}) \) are only approximately known and the number of \( \{ \delta_w, \delta_{ph} \} \) data couples used in this paper are 43 (see later). Thus, we may assume the Student’s t value equal to unit and write \( \sigma(\delta_w) \equiv s(\delta_w) \) and \( \sigma(\delta_{ph}) \equiv s(\delta_{ph}) \).

Let us consider a regression with \( X \) and \( Y \) as independent and dependent variable, respectively. Let us denote by \( \mu \) as the \( Y \) value at the point \( X \) of the population regression line. Since this line may be only estimated, we can only obtain an estimation, \( \hat{Y} \), of \( \mu \) by using the sample regression line. The estimated uncertainty of \( \hat{Y} \), \( u(\hat{Y}) \), which represents the uncertainty on the height, \( \mu \), of the experimental regression line at \( X \); is given by the following equation (Snedecor and Cochran, 1968):

\[
 u(\hat{Y}) \approx k(\alpha) S(\hat{Y}) = k(\alpha) \sqrt{\frac{s(yx)^2}{n} + \frac{x^2s(yx)^2}{\Sigma x^2}} = k(\alpha) s(yx) \sqrt{\frac{1}{n} + \frac{x^2}{\Sigma x^2}} \tag{1a}
\]

where \( n \) is the number of \( \{ \mathbf{X}, \mathbf{Y} \} \) couples of data used in the regression, \( s(yx) \) \( \equiv \sigma(yx) \), \( x = (X - X_m) \), \( X_m \) is the average of the values of the variable \( X \) used in the regression, \( k(\alpha) \) is the coverage factor for a normal distribution at \( \alpha \) significance level (\( k(\alpha) = 1 \) for \( \alpha = 0.32 \), \( k(\alpha) = 1.96 \) for \( \alpha = 0.05 \), and so on). Thus

\[
 \hat{Y} - u(\hat{Y}) \leq \mu \leq \hat{Y} + u(\hat{Y})
\]

Actually, we use the regression to estimate the individual true value \( Y \) corresponding to a new measurement \( X \). The obtained value is again \( \hat{Y} \), but its standard uncertainty is different and related to the difference \( \hat{Y} - Y \); thus, the prediction uncertainty of an individual \( Y \) value, \( u(Y) \), includes the uncertainty on the regression line as well as the uncertainty, \( s(yx) \), on an individual \( Y \):

\[
 u(Y) \approx k(\alpha) S(Y) = k(\alpha) \sqrt{s(yx)^2 + \frac{s(yx)^2}{n} + \frac{x^2s(yx)^2}{\Sigma x^2}} = k(\alpha) s(yx) \sqrt{1 + \frac{1}{n} + \frac{x^2}{\Sigma x^2}} \tag{1b}
\]

Thus

\[
 \hat{Y} - u(Y) \leq Y \leq \hat{Y} + u(Y)
\]

This means that there are \( (1 - \alpha) \) probability that the individual value of \( Y \) for a new \( X \) falls in the defined interval. Using this equation and varying \( X \), two curves are defined around the experimental regression line; they limit the prediction interval (P.I.) for a new observation.
Equations (1a) and (1b) are valid assuming that uncertainty on X is zero, that is usually not true. However, according to Taylor (1997, p. 190), the value $\sigma(X)$ may transferred to the uncertainty $\sigma(y|x)$ of Y; i.e., $\sigma(y|x)_\text{tot} = [\sigma(y|x)^2 + [B \sigma(X)]^2]^{0.5}$; thus $\sigma(y|x)_\text{tot} \approx s(y|x)_\text{tot} = [s(y|x)^2 + [B s(X)]^2]^{0.5}$. If where B is the slope of the regression line. Now equations (1a) and (1b) becomes

$$u(\hat{Y}) \approx k_{(\alpha)} s(y|x)_\text{tot} \sqrt{\frac{1}{n} + \frac{x^2}{\Sigma x^2}}$$ (1c)

and

$$u(Y) \approx k_{(\alpha)} s(y|x)_\text{tot} \sqrt{1 + \frac{1}{n} + \frac{x^2}{\Sigma x^2}}$$ (1d)

At last, it is very important to remember that uncertainties on $\delta$ and on $(\delta + 1)$ have the same value.

Oxygen isotope value in apatite phosphate group ($\delta_{ph}$) of humans, body water ($\delta_{bw}$) and presumed drinking ($\delta_{w}$)

Linear correlation between $\delta_{ph}$ (oxygen of the phosphate group of bioapatite) and $\delta_{bw}$ (oxygen of the body water) may been supposed a priori because we can reasonably assume equilibrium between oxygen of body water and phosphate group of biogenic apatite, that is confirmed, for instance, by the data on pigs (Longinelli, 1984) discussed before (paragraph 1). On the other hand, linear correlation between $\delta_{ph}$ and $\delta_{w}$, could be assumed a priori only in the case a model exists which relates linearly $\delta_{ph}$ and $\delta_{w}$.$\text{Actually, the models of Luz et al. (1984) and Bryant and Froelich (1995), under particular conditions predict linearity between } \delta_{bw} \text{ and delta values of totally ingested liquid water (} \delta_{lw} \text{). Rearranging these equations, the linear function (} \delta_{bw} + 1 \text{) = f(} \delta_{lw} + 1 \text{), where } \delta_{lw} \text{ refers to the total ingested liquid water, may be transformed into the linear relation (} \delta_{ph} + 1 \text{) = g(} \delta_{lw} + 1 \text{), and, approximately, in the linear relation (} \delta_{ph} + 1 \text{) = h(} \delta_{lw} + 1 \text{).}

From equation (7) of Bryant and Froelich (1995, p. 4526), we obtain the following steady-state mass-balance equation:

$$\delta_{bw} + 1 = b_1 (\delta_{lw} + 1) + b_0$$ (2)

The slope $b_1$ is the ratio between the input flux of total ingested liquid water and the sum of output flux of liquid water, CO$_2$ and water vapour; $b_0$ depends on the same parameters of $b_1$ and, in addition, on the $\delta^{18}$O of metabolic oxygen incorporated into the body and on the $\delta^{18}$O of oxygen uptaken in the lungs (uptaken O$_2$ is fractionated in respect to atmospheric O$_2$). Taking into account the fractionation coefficient, $\alpha_{ph/bw} = (\delta_{ph} + 1)/(\delta_{bw} + 1)$ between phosphate and body water, equation (2) becomes

$$(\delta_{ph} + 1) = \alpha_{ph/bw} b_1 (\delta_{lw} + 1) + \alpha_{ph/bw} b_0$$
As a first approximation, assuming for all the investigated humans a constant ratio \((\delta_w + 1)/(\delta_w + 1)\), where \(\delta_w\) is referred to the drinking water, we obtain an equation of the form \(Y = B \times X + A\), i.e.

\[
(\delta_{ph} + 1) = B (\delta_w + 1) + A
\]

where \(B\) and \(A\) may be calculated by regression of \((\delta_{ph} + 1)\) and \((\delta_w + 1)\) data from the literature. With the aim of defining equation (3), forty three couples of data, \({\delta_{ph}+1, \delta_w+1}\}, for humans have been collected from Longinelli (1984), Luz et al. (1984), Levinson et al. (1987) and Daux et al. (2008). Tests of normality (Table 2) are good for \((\delta_{ph}+1)\) and acceptable for \((\delta_w+1)\) (see Paragraph 2).

The obtained ordinary last-squares regression line (OLS) (equation 3) is reported in Table 3 together with other important statistical parameters. The most important preliminary tests concern the correlation coefficients, homoscedasticity, autocorrelation and normal distribution of residuals, the last one being very important for hypothesis tests and for confidence and prediction interval evaluation. All the parametric and non-parametric correlation coefficients (Pearson, Spearman, Kendall) are highly significant \((\alpha << 0.001)\), autocorrelation of residuals may be rejected \((p\text{-no-positive autocorrelation}) = 0.607, \text{Durbin-Watson’s test})\) homoscedasticity, however, is not high \((p\text{-homosc} = 0.105, \text{Breusch-Pagan’s test});\) the null hypothesis for the intercept, i.e. \(H_0: A = 0\), may be rejected at \(\alpha = 0.001\). The resulting regression line is the following:

\[
\delta_{ph} + 1 = 0.5414 (\delta_w + 1) + 0.4793
\]

or, alternatively,

\[
\delta_{ph} = 0.5414 \delta_w + 0.0306
\]

Since the intercept of equation (4a) is significantly different from zero, the ratio \((\delta_{ph} + 1)/(\delta_w + 1)\) cannot be regarded as a fractionation coefficient.

The \(R^2\) values for equation (4) is high \((0.841)\), thus indicating that the variation of the dependent variable is largely explained by the independent one and, through equation (2), that the role of the drinking water is the most important factor in determining the isotopic feature of the oxygen of the apatite phosphate group.

Both the uncertainty, \(u(\bar{Y})\), on \(\bar{Y}\) and prediction uncertainty, \(u(Y)\), for a new individual \(Y (= \delta_{ph} + 1)\), have been calculated assuming the standard deviation \(s(X)\) on \(\delta_w\) equal to 1‰ (see Luz et al., 1984). Results are reported in Table 3. The \(s(yx)\) and \(s(yx)_{tot}\) values are higher than the evaluated bibliographic standard uncertainties on \(\delta_{ph}\), which have a median of 0.4‰ (see Paragraph 2). This result was expected considering the complexity of the relation between \(\delta_w\) and \(\delta_{ph}\) discussed before and confirms significant
contribution to the ($\delta_{ph} + 1$) variance other than the uncertainty of the bibliographic ($\delta_{ph} + 1$). Results are graphically reported in Fig. 1.

![Fig. 1](image)

Fig. 1 $\delta_{ph} + 1$ and $\delta$ in ‰ for phosphate bioapatite and presumed water. The pointed lines define the confidence interval for $\hat{Y}$ and the dotted lines delimit the prediction interval for a new $Y$ value.

Estimation of $u(\hat{Y})$ and $u(Y)$ has been performed assuming $s(\delta_{w})$ = 1‰ in agreement with Luz et al. (1984) evaluation. The estimated standard uncertainty, on the response $\hat{Y}$ ($= \delta_{ph} + 1$) is about 0.3‰ ([Max+ Min]/2, see foot note of Table 3) with interval from 0.2‰ to 0.5‰. The prediction standard uncertainty, $u(Y)$, for an individual $Y$ ($= \delta_{ph} + 1$) is 1.3‰ (from 1.3‰ to 1.4‰). It is noteworthy that also large variation of $s(\delta_{w})$ do not change largely the uncertainty evaluation. For instance, for $s(\delta_{w}) = 0.5‰$, both uncertainty of $\hat{Y}$, $u(\hat{Y})$, and prediction uncertainty of an individual $Y$, decrease only of about 0.1‰ because of the small slope of the equations (4). This is very important for our calculation since, as stated before (see Paragraph 2), the value of $s(X)$ is not well known.

**Oxygen isotope in phosphate group of apatite ($\delta_{ph}$) used for water $\delta_{w}$ prediction**

As seen before, ($\delta_{ph} + 1$) of phosphate is strongly dependent on ($\delta_{w} + 1$) of water and the related uncertainties $u(\hat{Y})$ is minor than unit; in other words, according to the linear equation $Y = B \times X + A$, where $Y \equiv (\delta_{ph} + 1)$ and $X \equiv (\delta_{w} + 1)$, ($\delta_{ph} + 1$) could be considered as a satisfactory response to
the regressor \((\delta_w + 1)\). This, however, does not guarantee that \((\delta_{ph} + 1)\) is a satisfactory predictor of \((\delta_w + 1)\) for applications to archaeological sciences and environmental studies regarding the past.

Actually, phosphate of the bone and tooth bioapatite is frequently analysed in order to obtain information on past environmental water. This, however, may be done only in the case the uncertainty on the calculated new \(\delta_w\) values is small in comparison to the experimental range of \(\delta_w\) values. Moreover, if we assume that the estimated \(\delta_w\) has the same value of the local precipitation, \(\delta_{ph}\) could be also used to infer palaeoclimatic conditions. This assumption, however, may be misleading if no evidence exists supporting that local precipitation has similar delta value of drinking water. Discrepancy may occur, for instance, when the drinking water used derives from long rivers (e.g.: the Nile, in Africa), which are fed by precipitation occurring in areas far from that of interest.

There are two different ways to estimate \((\delta_w + 1)\) from \((\delta_{ph} + 1)\): (1) On the basis of the classical approach (Snedecor and Cochran, 1968; Parker et al., 2010, and reference therein), an estimation \(\hat{X}\) of \(X (\equiv \delta_w + 1)\) is obtained using the reverse equation \(X = (Y - A) / B\). (2) The value of \(\hat{X}\) may also be determined by inverse regression, i.e. considering \(X\) as dependent on \(Y\). According to Parker et al. (2010), the last approach is intuitively appealing; however, in this case bias seems to be higher than in the classical approach (Parker et al., 2010, Fig. 3 p. 337-338). In this paper, both the approaches have been used in processing the 43 data used above. The results are reported in Table 3 for the classical approach.

**Classical approach.** The linear equation becomes \(X = (Y/B) - A/B\), i.e.

\[
\delta_w + 1 = \frac{\delta_{ph} + 1}{B} - \frac{A}{B}
\]

and, thus, taking into account, the values of \(A\) and \(B\) reported in equations (4),

\[
\delta_w + 1 = \frac{((\delta_{ph} + 1) - 0.4793)/0.5414 = 1.8471 (\delta_{ph} + 1) - 0.8853}{5a}
\]

or, alternatively,

\[
\delta_w = 1.8471 \delta_{ph} - 0.0382 \quad 5b
\]

Granted that \(c^2 \cong k_s (s_{xy})^2_{tot}/\Sigma x^2\) (Snedecor and Cochran, 1968) is minor than 0.05 (Miller, 1993), the uncertainty may be calculated using equations (1) after substitution \(s(x)_{tot}/B\) for \(s(x)_{tot}\) and \((Y - Y_m)/B\) for \(x\) (Snedecor and Cochran, 1968), where \(B\) is the slope of equations (4). The obtained values of \(u(X) (\equiv u(\delta_w))\) (Table 3) is high, about 2.5\% at \(\alpha = 0.32\). It is noteworthy that differences in the isotope composition of local water less than 2.5\% (similar to our uncertainty on \(\delta_w\)) was already suggested by
Kirsanow and Tuross (2011) to be invisible in tissues values of archaeological, palaeontological and ecological interest.

**Inverse regression.** We calculated the OLS regression line \( X = B' Y + A' \), i.e.

\[
(\delta_w + 1) = B' (\delta_{ph} + 1) + A'
\]

and obtained

\[
(\delta_w + 1) = 1.5539 (\delta_{ph} + 1) - 0.5877
\]

(6a)

or, alternatively,

\[
\delta_w = 1.5539 \delta_{ph} - 0.0338
\]

(6b)

with intercept value significantly different from zero (Table 3).

Prediction uncertainty, \( u(X) (\equiv u(\delta_w)) \) has been calculated from the uncertainty on the median, 0.4‰, and the upper, 1.15‰, and lower, 0.2‰, values of \( s(Y) (\equiv s(\delta_{ph})). \) Also in this case, the prediction uncertainty is high, about 2.1 to 2.5‰ at \( \alpha = 0.32 \), and comparable to that obtained by the classical approach. The influence of the uncertainty \( s(Y) \) on \( u(X) \) is low to moderate as suggested by the differences between \( s(xy) \) and \( s(xy)_{\text{tot}} \) (from 1.97‰ to 2.27‰).

In spite equations (5) and (6) are apparently different, they lead to similar results in the limits of the uncertainty. For instance, two \( \delta_{ph} \) values are considered: one is 15.7‰, in the middle of the regression line, the other, 8.9‰, at the lower limit of the \( \delta_{ph} \) data. Actually, using equation (5) we obtain \( \delta_w \) values of -9.2‰ and -21.8‰ respectively; on the other hand, using equation (6), we obtain -9.4‰ and -20.0‰.

**Use of calculated \( \delta_w \) values for palaeotemperature evaluation**

Sometimes the \( \delta_w \) values calculated from \( \delta_{ph} \) (both for humans and animals) are used for palaeotemperature, \( T \), evaluation. It is noteworthy that the estimation of the prediction uncertainty on \( T \) must take into account uncertainty related to the equation used for the temperature computation

\[
T(\degree C) = C \delta_w + D,
\]

with standard deviation of residuals \( s(T\delta_w) \), as well as uncertainty \( S(\delta_w) \) (see equation 1b) related to the equation

\[
(\delta_w + 1) = [(\delta_{ph} + 1)/B] - (A/B)
\]

(7)

Thus, transferring \( S(\delta_w) \) to the \( T \) axis, we obtain

\[
s(T\delta_w)_{\text{tot}} = [s(T\delta_w)^2 + S(\delta_w)^2]^{0.5}
\]

At last, the calculated value \( s(T\delta_w)_{\text{tot}} \) would be used in equation (1d) to obtain the prediction uncertainty on \( T \) at different \( \delta_w \) values.

**Conclusion**

We can conclude recalling the question posed thirty years ago by Longinelli (1984) in the title of his pioneer paper: “Oxygen isotopes in
mammal bone phosphate: A new tool for palaeohydrological and palaeoclimatological research?” The answer, of course, depends on what we want. In the case \( \delta_{ph} \) is used for quantitative evaluation, the following points should be taken into consideration:

On the basis of the discussion reported in Paragraph 2, the calculated uncertainty \( u(\delta_w) \approx 2.5\% \) must be only considered as an approximate evaluation. Moreover, it is noteworthy that this value is a little underestimated because it has been calculated starting from \( \delta_{ph} \) data which, generally, are not single measurements but, mostly, arithmetic means of several measurements.

Consider \( u(\delta_w) \approx 2.5\% \) and compare two values obtained using equations (5), e.g. -12\% and -8.5\%, which are apparently largely different and could indicate significant temperature variation. The difference between these values is \( \Delta = |\delta_{w(1)} - \delta_{w(2)}| = (12 - 8.5)\% = 3.5\% \), the uncertainty on \( \Delta \) is \( u_\Delta = (2.4^2 + 2.4^2)^{0.5} = 3.5\% \) and thus \( \Delta/u_\Delta = 1 \). The value of this ratio give about 32% probability that \( \delta_{w(1)} \) and \( \delta_{w(2)} \) are different and, thus, we can conclude that only differences \( \Delta = |\delta_{w(1)} - \delta_{w(2)}| \) of the order of at least 3-4 delta unit \( \% \) may realistically assume an acceptable significance.

Uncertainty \( u(\delta_w) \) is reduced if we dispose of several (m) \( \delta_{ph} \) data for humans who were drinking the same water, a condition, however, which must be verified. Actually, in this case, in equation (1d), the value 1 under square roof is substituted by \( 1/m \).

The \( \delta_w \) and \( \delta_{ph} \) values used in the regression range from -21.7\% to -2\% and from 8.9 to 21.25\%, respectively; to avoid increase of uncertainty, no extrapolation from these ranges would be done.

Inference about the isotope values of meteoric water in the past could be made only if, for the period considered, the assumption meteoric water \( = \) drinking water at that time is supported by some evidence or assumed on the basis of reliable considerations.

The use of calculated \( \delta_w \) for temperature prediction must take into account not only the value \( s(T\delta_w) \), related to the function \( T(°C) = C \delta_w + D \), but also the value \( S(\delta_w) \) (see equation 1b) related to the function \( (\delta_w + 1) = [(\delta_{ph} + 1)/B] - (A/B) \), otherwise the total uncertainty could be underestimated. Unfortunately, at our knowledge, the values of \( s(T\delta_w) \) are never reported in the articles, thus making impossible a reliable evaluation of prediction uncertainty on the calculated \( T \).

Concluding, the answer to the Longinelli’s question could be yes only in the case the \( \delta_{ph} \) vs \( \delta_w \) relation is used with caution, always taking into consideration that the uncertainty on the calculated \( \delta_w \) is broad and that for palaeoclimatic studies it cannot be overlooked. A similar uncertainty evaluation would be performed also for other mammals.
Table 1. Symbols frequently used in the text and in Tables 2 and 3

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta )</td>
<td>Delta value: ( \frac{[^{18}\text{O}/^{16}\text{O}]<em>{\text{sample}}}{[^{18}\text{O}/^{16}\text{O}]</em>{\text{V-SMOW}}} - 1 )</td>
</tr>
<tr>
<td>( w, lw, ph )</td>
<td>Presumed drinking water, total ingested liquid water, phosphate group of biogenic apatite, respectively</td>
</tr>
<tr>
<td>( n )</td>
<td>Numbers of couples ( {X,Y} ) of isotopic data</td>
</tr>
<tr>
<td>( \alpha \alpha )</td>
<td>Significance level</td>
</tr>
<tr>
<td>( X_m, Y_m )</td>
<td>Arithmetic mean for ( X ) and ( Y ), respectively</td>
</tr>
<tr>
<td>( A, B ) or ( A', B' )</td>
<td>Intercept and slope of the calculated regression line, respectively</td>
</tr>
<tr>
<td>( s_A, s_B ) or ( s_A', s_B' )</td>
<td>Estimated standard error on the intercept and on the slope, respectively</td>
</tr>
<tr>
<td>( \sigma(X), \sigma(Y) )</td>
<td>Standard deviation of the ( X ) and ( Y ) population, respectively</td>
</tr>
<tr>
<td>( s(X), s(Y) )</td>
<td>Experimental standard deviation for the bibliographic data ( X ) and ( Y )</td>
</tr>
<tr>
<td>( \sigma(\text{yx}), \sigma(xy) )</td>
<td>Standard deviation of residuals on ( Y ) and ( X ), respectively</td>
</tr>
<tr>
<td>( s(\text{yx})<em>{\text{tot}}, s(\text{xy})</em>{\text{tot}} )</td>
<td>Experimental standard deviation of residuals on ( Y ) and ( X ), respectively</td>
</tr>
<tr>
<td>( \hat{X}, \hat{Y}, \hat{Y}_u, \hat{X}_u )</td>
<td>( X ) and ( Y ) estimated by regression lines, respectively</td>
</tr>
<tr>
<td>( S(\hat{X}), S(\hat{Y}) )</td>
<td>Standard uncertainty on the average response ( \hat{X} ) and ( \hat{Y} ), respectively</td>
</tr>
<tr>
<td>( u(\hat{X}), u(\hat{Y}) )</td>
<td>Uncertainty on the average response ( \hat{X} ) and ( \hat{Y} ), respectively</td>
</tr>
<tr>
<td>( S(X), S(Y) )</td>
<td>Prediction standard uncertainty for individual ( X ) and ( Y ), respectively</td>
</tr>
<tr>
<td>( u(X), u(Y) )</td>
<td>Prediction uncertainty for individual ( X ) and ( Y ), respectively</td>
</tr>
</tbody>
</table>

Table 2. Statistic results

<table>
<thead>
<tr>
<th>Number of data couples, ( n )</th>
<th>43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of ( \delta_w )</td>
<td>from (-21.7%) to (-2.0%)</td>
</tr>
<tr>
<td>Range of ( \delta_{ph} )</td>
<td>from (8.9%) to (21.3%)</td>
</tr>
<tr>
<td>Mean of ( \delta_w + 1 ) (± experimental standard deviation)</td>
<td>0.9906 (± 0.0049)</td>
</tr>
<tr>
<td>Mean of ( \delta_{ph} + 1 ) (± experimental standard deviation)</td>
<td>1.0157 (± 0.0029)</td>
</tr>
</tbody>
</table>

**Normality test for \( \delta_w + 1 \)**

- PPCCr (PPCCr*, \( \alpha = 0.05 \)) = 0.975 (0.974*)
- \( p \) (normal) \( W \) = 0.044
- \( p \) (normal) \( A \) = 0.126

**Normality test for \( \delta_{ph} + 1 \)**

- PPCCr (PPCCr*, \( \alpha = 0.05 \)) = 0.984 (0.974*)
- \( p \) (normal) \( W \) = 0.254
- \( p \) (normal) \( A \) = 0.220

Normality test: PPCCr = Probability Plot Correlation Coefficient (Looney & Gulledge, 1985) and PPCCr* = critical PPCCr at defined \( \alpha \). \( W \) = Shapiro and Wilk, \( A \) = Anderson-Darling.

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Table 3. Results of regression and inverted regression

<table>
<thead>
<tr>
<th></th>
<th>Regression (OSL)</th>
<th>Inverted regression (OSL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X = (δₜₚ+1), Y = ( δₚₚ+1)</td>
<td>Y = B X + A</td>
<td>X = (Y/B) - A/B</td>
</tr>
<tr>
<td>B (± sₜₚ) and B’ (± sₚₚ)</td>
<td>0.5414 (± 0.0405)</td>
<td>1.5539 (± 0.105)</td>
</tr>
<tr>
<td>A (± sₚₚ) and A’ (± sₚₚ)</td>
<td>0.4793 (± 0.0402)</td>
<td>-0.5877 (± 0.107)</td>
</tr>
<tr>
<td>1/B</td>
<td>1.8471</td>
<td></td>
</tr>
<tr>
<td>A/B</td>
<td>0.8853</td>
<td></td>
</tr>
<tr>
<td>R (p-uncorrelated)</td>
<td>0.917 (5.5*10⁻¹⁸)</td>
<td>0.917 (5.5*10⁻¹⁸)</td>
</tr>
<tr>
<td>rₛ (p-uncorrelated)</td>
<td>0.882 (5.9*10⁻¹⁵)</td>
<td>0.882 (5.9*10⁻¹⁵)</td>
</tr>
<tr>
<td>τ (p-uncorrelated)</td>
<td>0.740 (2.8*10⁻¹²)</td>
<td>0.740 (2.8*10⁻¹²)</td>
</tr>
<tr>
<td>Null hypothesis for the intercept</td>
<td>t &gt; tₜ (α = 0.001, df = 41)</td>
<td>t &gt; tₜ (α = 0.001, df = 41)</td>
</tr>
<tr>
<td>s(X) and s(Y)</td>
<td>0.0010</td>
<td>from 0.0002 to 1.15</td>
</tr>
<tr>
<td>s(yx) and s(xy)</td>
<td>0.00116</td>
<td>0.00196</td>
</tr>
<tr>
<td>s(yx)ₜₒₜ and s(xy)ₜₒₜ</td>
<td>0.00128</td>
<td>from 0.00197 to 0.00227*</td>
</tr>
<tr>
<td>Normality test for residuals, e</td>
<td>PPCCr (PPCCr*, α = 0.05)</td>
<td>0.988 (0.974*)</td>
</tr>
<tr>
<td></td>
<td>p-normal W</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>p-normal A</td>
<td>0.306</td>
</tr>
<tr>
<td>Homoscedasticity, Autocorrelation of residuals</td>
<td>p-homose BP</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>p-no autocorr DW</td>
<td>0.607</td>
</tr>
<tr>
<td>Uncertainty on the average response</td>
<td>u(Ŷ) (in %)</td>
<td>u(X) (in %)</td>
</tr>
<tr>
<td></td>
<td>u(X̂) (in %)</td>
<td>u(X) (in %)</td>
</tr>
<tr>
<td>Uncertainty range</td>
<td>from ±0.5 to ±0.5</td>
<td>from ±0.3 to ±1.0</td>
</tr>
<tr>
<td>Prediction uncertainty for individual X or Y</td>
<td>u(Y) (in %)</td>
<td>u(X) (in %)</td>
</tr>
<tr>
<td></td>
<td>from ±1.3 to ±1.4 (±1.3)</td>
<td>from ±2.4 to ±2.6 (±2.5)</td>
</tr>
</tbody>
</table>
e = residual. Correlation coefficient: R = Pearson, r = Spearman, \( \tau \) = Kendall. \( t^* \) = critical \( t \)-Student at defined \( \alpha \) and degree of freedom. Normality test: PPCCr = Probability Plot Correlation Coefficient (Looney & Gulledge, 1985) and PPCCr\(^*\) = critical PPCCr at defined \( \alpha \). W = Shapiro and Wilk, A = Anderson-Darling. Homoscedasticity test: BP = Breusch-Pagan; autocorrelation test: DW = Durbin-Watson. \( u(\bar{X}) \) and \( u(\bar{Y}) \) = uncertainty (\( \alpha = 0.32 \)) on the average response \( \bar{X} \) and \( \bar{Y} \), respectively; \( u(X) \) and \( u(Y) \) = prediction uncertainty (\( \alpha = 0.32 \)) for individual \( X \) and \( Y \), respectively. Max and Min = maximum and minimum uncertainty values. \# , values obtained assuming \( s(Y) \) equal to 0.2\% and to 1.1\%, respectively. ##, the data represent, in the order, the minimum value obtained for \( s(Y) = 0.2\% \), the maximum value obtained for \( s(Y) = 1.1\% \), and the (Max+Min)/2 value obtained for the median \( s(Y) = 0.40\% \).

**Acknowledgments**

The authors would like to thank Orazio Rossi (Professor of Multivariate Statistical Analysis, now retired) for his helpful suggestions on statistical analysis during the writing of this paper.

**References:**


