THE δ^{18}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 (δ_{ph}) in mammal bones and teeth is strongly correlated to that of the drinking water (δ_w) ingested by the mammal itself. However, the relation between (δ_{ph}) and (δ_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 δ_w calculated from a new value of δ_{ph} is on the order of 2.5‰. It means that only in the case the difference between two calculated δ_w values is higher than about 3-4‰, the δ_w values may be considered significantly different. This represents a big limit which cannot be underestimated in the use of human δ_{ph} for prediction of single δ_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 palaeclimatic 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], δ_{ph} ($\equiv \delta^{18}O_{ph}$), which is independent from the environmental temperature and that, for a given species, gives constant ratio $(\delta_{ph}+1)/(\delta_{bw}+1) = \boldsymbol{\alpha}_{ph/bw}$, where $\boldsymbol{\alpha}_{ph/bw}$ is the fractionation factor between the bioapatite phosphate group, ph, and the body water, bw, and δ_{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, $\boldsymbol{\alpha}_{ph/bw}$.

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 δ_{ph} and δ_w of the drinking water for different animal species.

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[4] According to the IUPAC (International Union of Pure and Applied Chemistry) recommendation, we consider $\delta = (R/R_{st}) - 1$, where R and R_{ST} 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 δ because it makes easier to recognise possible fractionation factors, $\boldsymbol{\alpha}$. Symbols frequently used are reported in Table 1.

A very important questionable point is the following: from δ_{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 δ_{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 (δ_w) and phosphate group of bone and tooth apatite (δ_{ph}) are significantly correlated, the δ_{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 (δ_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, $s(\delta_w)$, of δ_w 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 exactly 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 $\delta^{18}O$ 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 δ_{ph} 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 δ_{ph} 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 δ_{ph} vs δ_w because the authors frequently report only data which are averages of several measurements rather than the single measurements.

The experimental standard deviation for the δ_{ph} data reported by Longinelli (1984) ranges from 0.1_5‰ 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 1.1_5‰, 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 ($\alpha = 0.32$) around the true median M: $(0.40 - 0.20)‰ \leq M \leq (0.40 + 0.39)‰$. 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 δ_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 $\sigma(\delta_w) \cong s(\delta_w)$ and $\sigma(\delta_{ph}) \cong s(\delta_{ph})$.

Let us consider a regression with X and Y as independent and dependent variable, respectively. Let us denote by μ 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 μ by using the *sample regression line*. The estimated *uncertainty of $\hat{Y}$*, $u(\hat{Y})$, which represents the uncertainty on the height, μ, of the experimental regression line at X; is given by the following equation (Snedecor and Cochran, 1968):

$$u(\hat{Y}) \quad \cong k_{(\alpha)}\, S(\hat{Y}) = k_{(\alpha)} \sqrt{\frac{s(yx)^2}{n} + \frac{x^2 s(yx)^2}{\Sigma x^2}} = k_{(\alpha)}\, s(yx) \sqrt{\frac{1}{n} + \frac{x^2}{\Sigma x^2}} \tag{1a}$$

where n is the number of {X,Y} couples of data used in the regression, $s(yx) \cong \sigma(yx)$, $x = (X - X_m)$, 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 α 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:

$$u(Y) \quad \cong k_{(\alpha)}\; S(Y) = k_{(\alpha)} \sqrt{s(yx)^2 + \frac{s(yx)^2}{n} + \frac{x^2 s(yx)^2}{\Sigma x^2}} =$$

$$= k_{(\alpha)}\; s(yx) \sqrt{1 + \frac{1}{n} + \frac{x^2}{\sum x^2}} \tag{1b}$$

Thus

$$\hat{Y} - u(Y) \leq Y \leq \hat{Y} + u(Y)$$

This means that there are (1 - α) 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 $\sigma(yx)$ of Y; i.e., $\sigma(yx)_{tot} = [\sigma(yx)^2 + [B\ \sigma(X)]^2\}^{0.5}$; thus $\sigma(yx)_{tot} \cong s(yx)_{tot} = [s(yx)^2 + [B\ s(X)]^2\}^{0.5}$. If where B is the slope of the regression line. Now equations (1a) and (1b) becomes

$$u(\hat{Y}) \quad \cong k_{(\alpha)}\ s(yx)_{tot} \sqrt{\frac{1}{n} + \frac{x^2}{\Sigma x^2}} \tag{1c}$$

and

$$u(Y) \quad \cong k_{(\alpha)}\ s(yx)_{tot} \sqrt{1 + \frac{1}{n} + \frac{x^2}{\sum x^2}} \tag{1d}$$

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

Oxygen isotope value in apatite phosphate group (δ_{ph}) of humans, body water (δ_{bw}) and presumed drinking (δ_w)

Linear correlation between δ_{ph} (oxygen of the phosphate group of bioapatite) and δ_{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 δ_{ph} and δ_w, could be assumed *a priori* only in the case a model exists which relates linearly δ_{ph} and δ_w. Actually, the models of Luz et al. (1984) and Bryant and Froelich (1995), under particular conditions predict linearity between δ_{bw} and delta values of totally ingested liquid water (δ_{lw}). Rearranging these equations, the linear function $(\delta_{bw} + 1) = f(\delta_{lw} + 1)$, where δ_{lw} refers to the total ingested liquid water, may be transformed into the linear relation $(\delta_{ph} + 1) = g(\delta_{lw} + 1)$, and, approximately, in the linear relation $(\delta_{ph} + 1) = h(\delta_w + 1)$.

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

$$\delta_{bw} + 1 \qquad = b_1\ (\delta_{lw} + 1) + b_o \tag{2}$$

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_o 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, $\boldsymbol{\alpha}_{ph/bw}$, $= (\delta_{ph} + 1)/(\delta_{bw} + 1)$ between phosphate and body water, equation (2) becomes

$$(\delta_{ph} + 1) \qquad = \boldsymbol{\alpha}_{ph/bw}\ b_1\ (\delta_{lw} + 1) + \boldsymbol{\alpha}_{ph/bw}\ b_o$$

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

$$(\delta_{ph} + 1) = B\,(\delta_w + 1) + A \qquad (3)$$

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 (δ_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(no-positive autocorrelation) = 0.607, Durbin-Watson's test) homoscedasticity, however, is not high (p-homosc = 0.105, Breusch-Pagan's test); the null hypothesis for the intercept, i.e. H_o: A = 0, may be rejected at $\alpha = 0.001$. The resulting regression line is the following:

$$\delta_{ph} + 1 = 0.5414\,(\delta_w + 1) + 0.4793 \qquad (4a)$$

or, alternatively,

$$\delta_{ph} = 0.5414\,\delta_w + 0.0306 \qquad (4b)$$

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, $u(\hat{Y})$, on $\hat{Y}$ and prediction uncertainty, u(Y), for a new individual Y ($\equiv \delta_{ph} + 1$), have been calculated assuming the standard deviation s(X) on δ_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 δ_{ph}, which have a median of 0.4‰ (see Paragraph 2). This result was expected considering the complexity of the relation between δ_w and δ_{ph} discussed before and confirms significant contribution to the (δ_{ph} +1) variance other than the uncertainty of the bibliographic (δ_{ph} + 1). Results are graphically reported in Fig. 1.

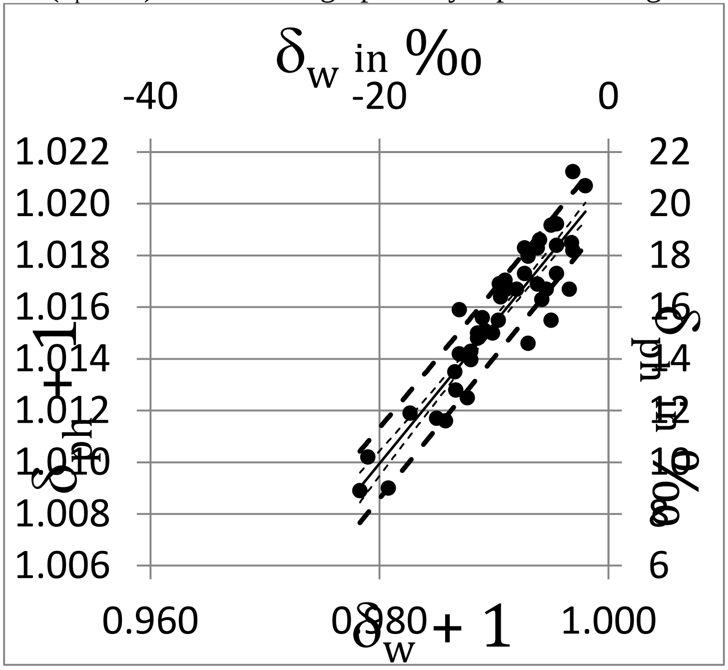

Fig.1 δ+1 and δ in ‰ for phosphate bioapatite and presumed water. The pointed lines define the confidence interval for Ŷ and the dotted lines delimite the prediction interval for a new Y value.

Estimation of u(Ŷ) and u(Y) has been performed assuming s(δ_w) =1‰ in agreement with Luz et al. (1984) evaluation. The estimated standard uncertainty, on the response Ŷ (≡ $\widehat{\delta_{ph} + 1}$) is about 0.3_5‰ ([Max+ Min]/2, see foot note of Table 3) with interval from 0.2‰ to 0.5_5‰. The prediction standard uncertainty, u(Y), for an individual Y (≡ δ_{ph} + 1) is 1.3_5‰ (from 1.3‰ to 1.4‰). It is noteworthy that also large variation of s(δ_w) do not change largely the uncertainty evaluation. For instance, for s(δ_w) = 0.5‰, both uncertainty of Ŷ, u(Ŷ), 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 (δ_{ph}) used for water δ_w prediction

As seen before, (δ_{ph} + 1) of phosphate is strongly dependent on (δ_w + 1) of water and the related uncertainties u(Ŷ) is minor than unit; in other words, according to the linear equation Y = B X + A, where Y ≡ (δ_{ph} + 1) and X ≡ (δ_w + 1), (δ_{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 δ_w values is small in comparison to the experimental range of δ_w values. Moreover, if we assume that the estimated δ_w has the same value of the local precipitation, δ_{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 $\widehat{X}$ of X ($\equiv \delta_w + 1$) is obtained using the reverse equation $X = (Y - A) / B$. (2) The value of $\widehat{X}$ 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.

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

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

$$(\delta_w + 1) = ((\delta_{ph} + 1) - 0.4793)/0.5414 = \\ = 1.8471\ (\delta_{ph} + 1) - 0.8853 \qquad (5a)$$

or, alternatively,

$$\delta_w = 1.8471\ \delta_{ph} - 0.0382 \qquad (5b)$$

Granted that $c^2 \cong k_{(\alpha)}\ s(xy)^2_{tot} / \Sigma x^2$ (Snedecor and Cochran, 1968) is minor than 0.05 (Miller, 1993), the uncertainty may be calculated using equations (1) after substitution $s(yx)_{tot}/B$ for $s(yx)_{tot}$ 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 δ_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.

$$(\delta_w + 1) \quad = B'\,(\delta_{ph} + 1) + A'$$

and obtained

$$(\delta_w + 1) \quad = 1.5539\,(\delta_{ph} + 1) - 0.5877 \qquad (6a)$$

or, alternatively,

$$\delta_w \quad = 1.5539\,\delta_{ph} - 0.0338 \qquad (6b)$$

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

Prediction uncertainty, u(X) (≡ u(δ_w)) has been calculated from the uncertainty on the median, 0.4‰, and the upper, 1.1$_5$‰, and lower, 0.2‰, values of s(Y) (≡ s(δ_{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)$_{tot}$ (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 δ_{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 δ_{ph} data. Actually, using equation (5) we obtain δ_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 δ_w values for palaeotemperature evaluation

Sometimes the δ_w values calculated from δ_{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

$$T(°C) \quad = C\,\delta_w + D,$$

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

$$(\delta_w + 1) \quad = [(\delta_{ph} + 1)/B] - (A/B) \qquad (7)$$

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

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

At last, the calculated value s(Tδ_w)$_{tot}$ would be used in equation (1d) to obtain the prediction uncertainty on T at different δ_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 δ_{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 δ_{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 Δ is $u_\Delta = (2.4^2 + 2.4^2)^{0.5} = 3.5$‰ and thus $\Delta/u_\Delta = 1$. The value of his 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) δ_{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 δ_w and δ_{ph} values used in the regression range from -21.7‰ to -2‰ and from 8.9 to 21.2_5‰, 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 δ_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 δ_{ph} vs δ_w relation is used with caution, always taking into consideration that the uncertainty on the calculated δ_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

δ	Delta value: $[(^{18}O/^{16}O)_{sample} / (^{18}O/^{16}O)_{V\text{-}SMOW})] -1$
w, lw, ph	Presumed drinking water, total ingested liquid water, phosphate group of biogenic apatite, respectively
n	Numbers of couples {X,Y} of isotopic data
$\alpha\alpha$	Significance level
X_m, Y_m	Arithmetic mean for X and Y, respectively
A, B or A', B'	Intercept and slope of the calculated regression line, respectively
s_A, s_B or $s_{A'}$, $s_{B'}$	Estimated standard error on the intercept and on the slope, respectively
e	Residuals
$\sigma(X)$, $\sigma(Y)$	Standard deviation of the X and Y population, respectively
s(X), s(Y)	Experimental standard deviation for the bibliographic data X and Y
$\sigma(yx)$, $\sigma(xy)$	Standard deviation of residuals on Y and X, respectively
s(yx), s(xy)	Experimental standard deviation of residuals on Y and X, respectively
$s(yx)_{tot}$, $s(xy)_{tot}$	$\{s(yx)^2 + B\ s(X)^2\}^{0.5}$, $\{s(xy)^2 + B'\ s(Y)^2\}^{0.5}$, respectively, and similar (see text)
$\hat{X}$, $\hat{Y}$ $\hat{Y}$,	X and Y estimated by regression lines, respectively
$S(\hat{X})$, $S(\hat{Y})$	Standard uncertainty on the average response $\hat{X}$ and $\hat{Y}$, respectively
$u(\hat{X})$, $u(\hat{Y})$	Uncertainty on the average response $\hat{X}$ and $\hat{Y}$, respectively
S(X), S(Y)	Prediction standard uncertainty for individual X and Y, respectively
u(X), u(Y)	Prediction uncertainty for individual X and Y, respectively

Table 2. Statistic results

Number of data couples, n	43
Range of δ_w	from -21.7‰ to -2.0‰
Range of δ_{ph}	from 8.9‰ to 21.3‰
Mean of $\delta_w + 1$ (± experimental standard deviation)	0.9906 (± 0.0049)
Mean of $\delta_{ph} + 1$ (± experimental standard deviation)	1.0157 (± 0.0029)
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 α. W = Shapiro and Wilk, A = Anderson-Darling.

Table 3. Results of regression and inverted regression

	Regression (OSL)		***Inverted regression (OSL)***
X = (δ_w+1), Y = (δ_{ph}+1)	Y = B X + A	X = (Y/B) - A/B	X = B' Y + A'
B (± s_B) and B' (± $s_{B'}$),	0.5414 (± 0.0405)		1.5539 (± 0.105)
A (± s_A) and A' (± $s_{A'}$)	0.4793 (± 0.0402)		-0.5877 (± 0.107)
1/B		1.8471	
A/B		0.8853	
R (p-uncorrelated)	0.917 ($5.5*10^{-18}$)		0.917 ($5.5*10^{-18}$)
r_s (p-uncorrelated)	0.882 ($5.9*10^{-15}$)		0.882 ($5.9*10^{-15}$)
τ (p-uncorrelated)	0.740 ($2.8*10^{-12}$)		0.740 ($2.8*10^{-12}$)
Null hypothesis for the intercept	$t > t^*_{(\alpha = 0.001, df = 41)}$		$t > t^*_{(\alpha = 0.001, df = 41)}$
s(X) and s(Y)	0.0010		from 0.0002 to 1.1_5
s(yx) and s(xy)	0.00116		0.00196
$s(yx)_{tot}$ and $s(xy)_{tot}$	0.00128		from 0.00197 to $0.00227^{\#}$
Normality test for residuals, e			
PPCCr (PPCCr*, α = 0.05)	0.988 (0.974*)		0.977 (0.974*)
p-normal W	0.356		0.094
p-normal A	0.306		0.042
Homoscedasticity, Autocorrelation of residuals			
p-homosc BP	0.105		0.923
p-no autocorr DW	0.607		0.748
Uncertainty on the average response $\hat{X}$ or $\hat{Y}$	u($\hat{Y}$) (in ‰)	u($\hat{X}$) (in ‰)	u($\hat{X}$) (in ‰)
Uncertainty range	from ±0.2 to $\pm 0.5_5$	from $\pm 0.3_5$ to ±1.0	from ±0.3 to ±0.8
Prediction uncertainty for individual X or Y	u(Y) (in ‰)	u(X) (in ‰)	u(X) (in ‰)
Uncertainty range, ([Max+Min]/2)	from ±1.3 to ±1.4 ($\pm 1.3_5$)	from ±2.4 to ±2.6 (±2.5)	from 2.0 to 2.5 $(\pm 2.1)^{\#\#}$

e = residual. Correlation coefficient: R = Pearson, r_s = Spearman, τ = Kendall. t* = critical t-Student at defined α and degree of freedom. Normality test: PPCCr = Probability Plot Correlation Coefficient (Looney & Gulledge, 1985) and PPCCr* = critical PPCCr at defined α. W = Shapiro and Wilk, A = Anderson-Darling. Homoscedasticity test: BP = Breusch-Pagan; autocorrelation test: DW = Durbin-Watson. $u(\hat{X})$ and $u(\hat{Y})$ = uncertainty ($\alpha = 0.32$) on the average response $\hat{X}$ and $\hat{Y}$, 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_5‰, 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_5‰, and the (Max+Min)/2 value obtained for the median s(Y) = 0.40‰,

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