



Probing dietary change of the Kwäday Dän Ts'inchí individual, an ancient glacier body from British Columbia: II. Deconvoluting whole skin and bone collagen $\delta^{13}\text{C}$ values via carbon isotope analysis of individual amino acids

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ABSTRACT

The remains of the Kwäday Dän Ts'inchí individual, a frozen male human, were recovered from a retreating glacier within the Tatshenshini-Elsek Park in British Columbia in August 1999. In order to provide information on both the geographical origin of this individual and how long he spent in the remote interior region prior to his death, molecular analysis and compound-specific carbon isotope analyses were performed on individual amino acids purified from his skin and bone. Gas chromatographic quantification of constituent amino acids of both tissues revealed a molecular distribution characteristic of collagen, dominated by glycine and to a lesser extent proline, hydroxyproline and alanine. Chiral gas chromatography indicated that protein preservation in both tissues was exceptional. Carbon isotope analysis of a faunal assemblage from an earlier prehistoric site from southern British Columbia provided reference dietary amino acid $\delta^{13}\text{C}$ values for terrestrial (deer and domestic dog) and marine species (salmon and sealion), showing clear separation in all amino acids, particularly glycine which was extremely ^{13}C -enriched in the marine animals. The distinction between terrestrial and marine organisms was increased by exploring $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values ($6.6 \pm 0.6\text{‰}$ and $15.0 \pm 2.1\text{‰}$ respectively), which were higher in the latter by approximately 8‰ , mirroring the increased $\delta^{15}\text{N}_{\text{Bulk collagen}}$ values observed for the marine animals ($R^2 = 0.78$; $p < 0.001$). The Kwäday Dän Ts'inchí individual's bone had a similarly elevated $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ value of $15.6 \pm 1.0\text{‰}$, indicating his extreme reliance on marine dietary resources throughout early life. The skin amino acid $\delta^{13}\text{C}$ values were consistently lower than those observed for bone, with a concurrently lower $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ value of $12.7 \pm 0.9\text{‰}$. The shift between the carbon isotope composition of bone (long-term diet) and skin amino acids (short-term diet) confirmed a sudden divergence away from marine food sources in the last months of life, consistent with his discovery 80 km inland.

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1. Introduction

The stable carbon and nitrogen isotope composition of body proteins has long been exploited to trace the dietary history of individual humans as well as human populations, through discrete differences between, for example, C_3 and C_4 terrestrial diets or terrestrial and marine diets (DeNiro, 1987; Koch et al., 1994; Price, 1989; Schoeninger, 1989; van der Merwe and Vogel, 1978; van der Merwe, 1982). Moreover, stable isotope compositions can also be employed as a forensic tool, in establishing a travelogue of an

individual based upon geographical dietary isotope variability, which is recorded in the stable isotope compositions of consumer proteins (Cerling et al., 2003; Ehleringer, 2005; Fraser et al., 2006; Roy et al., 2005). In this paper we describe the application of compound-specific carbon isotope analysis of individual protein amino acids as a palaeoforensic tool to investigate the diet, life, history and origin of this individual discovered in British Columbia. The frozen corpse of a young adult male was discovered in August 1999 projecting out of a melting glacier in the Tatshenshini-Elsek Park of northwestern British Columbia (59°N Latitude, 137°W Longitude (Beattie et al., 2000; Dickson et al., 2004; Pringle, 2002)). The discovery was designated Kwäday Dän Ts'inchí, i.e. "long ago person found" by the Champagne and Aishihik First Nations (CAFN)

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inhabitants of the region. Situated at an elevation of approximately 1600 m, the discovery site is located approximately 80 km from the strongly indented coast. A range of artefacts were discovered in association with the corpse, including a robe style fur garment, a plant fibre hat and various wooden artefacts. Initial radiocarbon dating indicated an age of 500 ± 30 BP for his clothing (Beattie et al., 2000); however, more recent radiocarbon dating of the body itself indicated a date between 1670 and 1850 cal AD, predating European contact in this region (Richards et al., 2007). The overriding question surrounding this individual was where he had originated and how long he spent in this glacial region prior to his death. It is possible that the Kwäday Dän Ts'inchí individual originated from: (i) one of the Southern Tutchone peoples inhabiting the interior region of the Tatshenshini Basin, or (ii) a coastal community, such as one of the Tlingit peoples of the Klukwan/Haines areas. If the latter was the case, it is important to ascertain how long he was in this glacial region of the interior prior to his death (Beattie et al., 2000; Dickson et al., 2004; Monsalve et al., 2002; Mudie et al., 2005; Pringle, 2002).

To address these questions, bulk carbon and nitrogen isotope analysis of a sample of bone collagen was performed initially, supporting a substantial marine diet (-13.7‰ and $+17.9\text{‰}$, respectively), in agreement with the known reliance of prehistoric coastal British Columbian populations on marine foods such as Pacific salmon (Chisholm et al., 1983, 1982; Lovell et al., 1986). The isotopic composition along a strand of preserved hair provided a less robust dietary signature due to its fragmentary nature, pointing towards a possible shift towards a more terrestrial/freshwater dietary profile in the last year of life (Richards et al., 2007). Both the molecular and carbon isotopic composition of individual lipids extracted from skin and bone samples indicated a diet dominated by marine foods (bone lipids) in the last year of life followed by a transition to a more terrestrial diet in the very latter period (skin lipids; Corr et al., 2008). In order to validate this conclusion, the objective here was to concentrate on protein amino acids in the bone and skin, as a further means of comparing long-term (bone: 10–30 years; Rucklidge et al., 1992) and short-term diet (skin: months; Gerber and Altman, 1960). The rationale is that the consumption of a high marine protein diet is likely to result in a shift from *de novo* synthesis of non-essential amino acids towards an increase in the direct assimilation of dietary amino acids for collagen synthesis (Corr et al., 2005; Jones, 2002; Schwarcz, 2001). The potential of using amino acid $\delta^{13}\text{C}$ values to identify high marine protein consumers, specifically by investigating $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values, has been previously demonstrated (Corr et al., 2005). However, compound-specific carbon isotope analysis of human protein amino acids represents an emerging technique in palaeodietary reconstruction and there is a shortage of reported reference dietary (i.e. meat, plant, fish etc.) amino acid $\delta^{13}\text{C}$ values. To overcome this we also determined $\delta^{13}\text{C}$ values for the amino acids of marine and terrestrial species from a faunal assemblage excavated at the prehistoric archaeological site of Dionisio Point, Galiano Island, British Columbia.

2. Materials and methods

All reagents and solvents used were, respectively, analytical or HPLC grade or better. Derivatizing agents were all from the same batch. Rigorous employment of analytical blanks was practiced throughout to monitor for the introduction of laboratory contamination.

2.1. Preparation of archaeological collagen

Bone and skin tissue samples of the Kwäday Dän Ts'inchí individual and the faunal bone samples from Dionisio Point were

surface-drilled to remove exogenous contaminants and ground using a Spex mill (SPEX CentriPrep, New Jersey, USA). The powdered samples were demineralised (0.5 M HCl, 5°C , 48 h), and the resultant solid was gelatinised (0.001 M HCl, 70°C , 48 h) in sealed tubes. The resulting collagen (gelatin) was then filtered through 30 kDa ultrafilters and the >30 kDa fraction lyophilised.

2.2. Amino acid hydrolysis

Approximately 2 mg of collagen was hydrolysed under vacuum in Young's tubes (6 M HCl; 100°C , 24 h). On cooling the samples were transferred to screw-capped vials with double distilled water (3×0.5 ml) and methanol (3×0.5 ml). The solutions were evaporated under a gentle stream of N_2 , re-dissolved in 2 ml of methanol and stored at -18°C until required for analysis.

2.3. Preparation of amino acid trifluoroacetyl isopropyl (TFA-IP) derivatives

An aliquot of each protein hydrolysate was transferred to a screw-capped reaction tube and dried under N_2 . γ -Amino-*n*-butyric acid ($40 \mu\text{l}$, 0.2 mg ml^{-1} solution in 0.1 M HCl) was added to each tube as an internal standard. Acidified isopropanol (0.5 ml, 2.8 M with acetyl chloride) was added to each reaction tube, which was sealed with a Teflon-lined cap and heated (100°C , 1 h). The reaction was terminated by placing the tubes in a freezer and the residual isopropanol was removed under a gentle stream of N_2 at 40°C . Excess H_2O and isopropanol were removed by addition of dichloromethane (DCM, 2×0.25 ml) which was also evaporated under N_2 . Trifluoroacetic anhydride (TFAA, 0.5 ml) and DCM were added to the reaction tubes, which were sealed with Teflon-lined caps and heated (100°C , 10 min). The tubes were then placed in an ice bath where the excess TFAA and DCM were removed under a gentle stream of N_2 . The derivatised amino acid trifluoroacetyl-isopropyl (TFA-IP) ester derivatives were dissolved in 0.25 ml of DCM and stored at -18°C until required for GC/C/IRMS analysis.

2.4. Instrumental analyses

2.4.1. Gas chromatography (GC)

GC analyses were performed on a Hewlett Packard 5890 series II gas chromatograph. Samples were introduced via an on-column injector equipped with an appropriate fused silica capillary column. Hydrogen was used as the carrier gas. Flame ionisation detection (FID) was used to monitor the column effluent and data were acquired and analysed using HP Chemstation software. Amino acids were analysed on a polar BPX-70 capillary column (SGE; $50 \text{ m} \times 0.32 \text{ mm id}$; $0.2 \mu\text{m}$ film thickness) coated with a 70% cyanopropyl polysilphenylene-siloxane stationary phase. The oven temperature programme was as follows: initial temperature was held isothermally at 40°C (1 min) and then increased to 120°C at $10^\circ\text{C min}^{-1}$, to 190°C at 3°C min^{-1} , and finally to 250°C (20 min) at 5°C min^{-1} . Chiral GC was performed on a Chirasil-Valine GC capillary column (Altech; diamide linked to polysiloxane, $50 \text{ m} \times 0.32 \text{ mm id}$; $0.2 \mu\text{m}$ film thickness). The oven temperature programme was as follows: initial temperature was held isothermally at 40°C (3 min) and then increased to 90°C at $45^\circ\text{C min}^{-1}$, and finally to 190°C (20 min) at 2°C min^{-1} .

2.4.2. Gas chromatography/mass spectrometry (GC/MS)

GC/MS analyses were performed on a ThermoFinnigan Trace GC/MS. Samples were introduced using a PTV injector set to splitless mode. Appropriate capillary columns and temperature programmes were utilised as described above. The MS was run in EI mode with a GC interface temperature of 300°C and a source temperature of 200°C ; the quadrupole MS scanned in the range

m/z 50–850 at 1.3 scans s^{-1} . The carrier gas was helium. The mass spectrometer was operated with an electron voltage of 70 eV. Data acquisition and processing were carried out using Xcalibur software.

2.4.3. Gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS)

Amino acid $\delta^{13}C$ value determinations were performed in triplicate following derivatisation to TFA-IP esters using a HP 6890 gas chromatograph coupled to a Finnigan MAT DELTA^{plus} XL isotope ratio monitoring mass spectrometer, via a Finnigan MAT GC III combustion interface (electron ionisation 100 eV, three Faraday cup collectors m/z 44, 45 and 46, CuO/NiO/Pt combustion reactor set to 940 °C, Cu reduction reactor set to 600 °C) as described in Howland et al. (2003). The GC was fitted with a ZB-5ms column (Zebtron; 60 m \times 0.32 mm, 0.25 μ m film thickness, 5% phenyl, 95% dimethylpolysiloxane equivalent stationary phase). The split-splitless injector temperature was maintained at 200 °C. Co-injected standards of alanine, phenylalanine and lysine TFA-methyl esters (Sigma-Aldrich products T3381, T5006 and T4631) of known isotopic composition were added to each sample immediately prior to GC/C/IRMS analysis to monitor instrument performance. Amino acid $\delta^{13}C$ values and associated errors were calculated from the $\delta^{13}C$ values of their TFA-IP derivatives using correction factors (Docherty et al., 2001; Jim et al., 2003; Silfer et al., 1991). Error propagation was employed to combine both the uncertainty associated with the use of correction factors to account for the kinetic isotope effect and the additional carbon atoms introduced during derivatisation. Carbon isotope ratios are reported relative to the V-PDB standard in parts per thousand (‰). Results are expressed as:

$$\delta^{13}C = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where R_{sample} and R_{standard} are the ratios of $^{13}C/^{12}C$ for the sample and the standard, respectively.

3. Results and discussion

3.1. Tissue amino acid compositions

Fig. 1(a) and (b) display partial gas chromatograms of the total hydrolysable amino acids from the bone and skin samples, respectively, analysed as trifluoroacetyl-isopropyl esters. The similarity of the amino acids present and their distributions may be attributed to the fact that both bone and skin protein is dominated by Type I collagen. The protein composition of skin, although dominated by collagen (ca. 71.9%), also includes a minor elastin (0.6–2.1%; lower hydroxyproline abundance) and an even smaller keratin (<1%; lower glycine abundance) component. Fig. 2 illustrates the relative abundances of amino acids detected in the bone and skin samples, in relation to their known distribution in modern human Type I collagen (Herring, 1972). Both distributions are very similar to that observed in the modern, undegraded collagen, indicating the remarkable level of protein preservation in these ancient tissues. The only exception is a somewhat lower abundance of the collagen-dominating amino acid glycine in the Kwāday Dān Ts'inchī individual's bone (27.3%) and skin (25.9%) samples compared to that of modern collagen (35.3%). This divergence may be explained in the higher susceptibility of glycine to degradation in archaeological deposits relative to other collagen amino acids; indeed, even higher relative losses of glycine are not uncommon in archaeological collagens (Tuross et al., 1988).

Further assessment of the integrity and indigeneity of the bone and skin amino acids was achieved using chiral gas chromatography to monitor the possible presence of D-enantiomers. Although amino acids can exist as L- or D-enantiomers, in nature L-amino

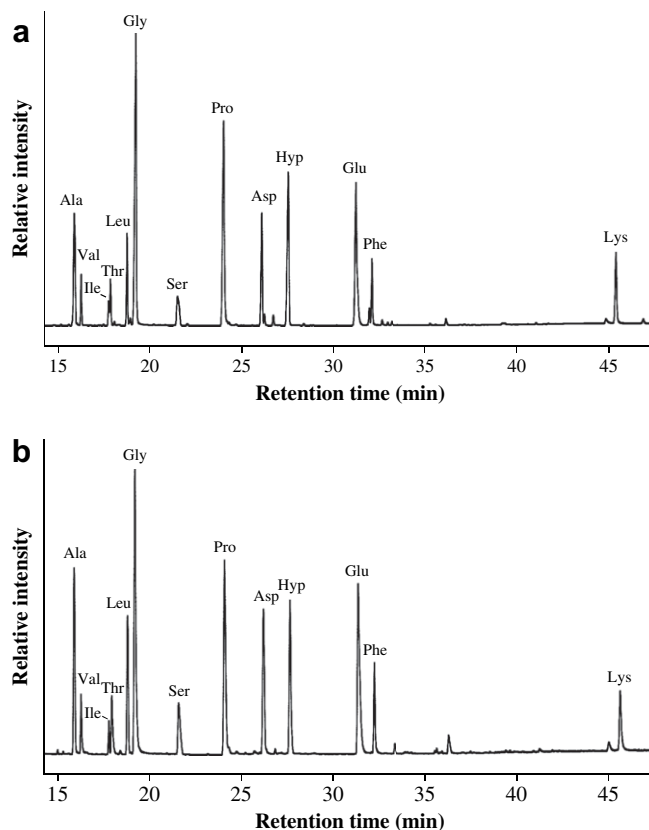


Fig. 1. Gas chromatograms of trifluoroacetyl-isopropyl (TFA-IP) esters of amino acids extracted from samples of the Kwāday Dān Ts'inchī individual's (a) bone and (b) skin.

acids are almost exclusively employed for protein biosynthesis. The occurrence of D-amino acids in archaeological samples results exclusively from: (i) abiotic racemisation or stereochemical inversion at the chiral centre in the conversion of L- to D-amino acids, and/or (ii) microbiological transformation of protein amino acids by bacteria which utilise D-amino acids (primarily D-alanine, D-aspartic

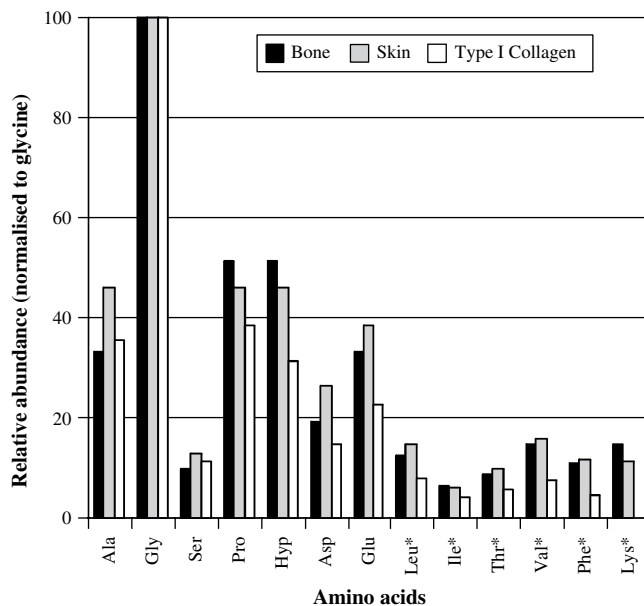


Fig. 2. The amino acid composition of bone and skin sampled from the Kwāday Dān Ts'inchī individual, quantified as TFA-IP esters using GC, in relation to the amino composition of human Type I collagen (Herring, 1972). * denotes essential amino acid.

acid and D-glutamic acid) in the synthesis of the cell wall polymer peptidoglycan (Madigan, 2003). Chiral GC revealed very minor abundances of D-alanine, D-aspartate, and D-glutamate in both the bone and skin samples. A slightly elevated abundance of D-alanine (D/L ratio: 0.006 and 0.003), D-aspartate (D/L ratio: 0.026 and 0.022) and D-glutamate (D/L ratio: 0.008 and 0.007) was detected in bone compared to skin. For each of the 3 amino acids the D-enantiomer was detected at abundances above those resulting from the racemisation of L- to D-amino acids known to be produced during acid hydrolysis of proteins (accounting for approximately 3–7% of D-enantiomers; Csapo et al., 1997). Since D-alanine, D-aspartic acid and D-glutamic acid are primary constituents of peptidoglycan their detection in the Kwäḏay Dän Ts'incẖ individual's tissues can be explained by very low level microbial activity in the skin and bone. D-enantiomers of these three amino acids exclusively are detectable in considerably higher abundance in well-preserved archaeological bone (D/L ratio: 0.019–0.025) and in much higher abundance in poorly-preserved bone (D/L ratio: 0.084–0.159; Elster et al., 1991).

3.2. Stable isotope composition of Dionisio Point faunal amino acids

Compound-specific stable isotope analysis was performed on bone collagen amino acids extracted from a range of faunal samples from the prehistoric site of Dionisio Point, located on Galiano Island, British Columbia. Deer ($n = 3$), dog ($n = 3$), salmon ($n = 3$) and sealion ($n = 1$) were selected to provide amino acid $\delta^{13}\text{C}$ values representative of both terrestrial and marine diets. The results of bulk collagen nitrogen isotope analysis ($+15.2 \pm 0.8\text{‰}$, $n = 3$) indicated that the dogs consumed a primarily marine diet, possibly derived from the scraps of the meals of humans with a reliance on a marine source of protein. Where human burials are lacking at archaeological sites dog collagen has been employed as an analogue for human collagen for stable isotope palaeodietary investigations because of their similar diets, derived directly or indirectly from humans (Cannon et al., 1999).

Amino acid $\delta^{13}\text{C}$ values and bulk collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for all specimens analysed in this research are presented in Table 1. The $\delta^{13}\text{C}$ values of the 8 most abundant amino acids in bone collagen (accounting for 84.8% of the carbon in collagen) of the faunal assemblage from Dionisio Point are presented in Fig. 3. A highly uniform range of inter-amino amino acid $\delta^{13}\text{C}$ values ($18.8 \pm 2.8\text{‰}$) was observed for the faunal species analysed, resulting from both the differing biochemical pathways involving amino acids and metabolically-associated isotopic fractionations. There is clear separation between the C_3 -terrestrial diet consuming deer and the marine animals (salmon and sealion) and domestic dogs with respect to all amino acids, both essential (denoted with an asterisks) and non-essential. The mean deer essential and non-essential amino acid $\delta^{13}\text{C}$ values (-22.4 and -20.5‰ , respectively) are lower by approximately 10‰ than those observed for marine fauna (-12.6 and -12.2‰ , respectively) and dogs (-11.4 and -11.1‰ , respectively). This significant separation between C_3 terrestrial and marine-based faunal species' $\delta^{13}\text{C}$ values reinforces the validity and integrity of the approach we employ to extract and derivatise amino acids from degraded archaeological proteins.

3.3. Stable isotope composition of the Kwäḏay Dän Ts'incẖ individual's bone collagen amino acids

Fig. 3 illustrates the Kwäḏay Dän Ts'incẖ individual's bone collagen amino acid $\delta^{13}\text{C}$ values in conjunction with the results obtained for the Dionisio Point faunal species. It is evident that all amino acid $\delta^{13}\text{C}$ values for the Kwäḏay Dän Ts'incẖ individual are aligned to those observed for the marine fauna and dogs and are well separated from the C_3 -terrestrial deer samples. This observation also provides support for using the domestic dog amino acid

Table 1
Bone collagen amino acid $\delta^{13}\text{C}$ values and bulk bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the faunal assemblage from Dionisio Point and the Kwäḏay Dän Ts'incẖ individual's skin and bone

	Ala	Gly	Thr	Ser	Val	Leu	Ile	Pro	Hyp	Asp	Glu	Phe	$\delta^{13}\text{C}_{\text{Bulk}}$	$\delta^{15}\text{N}_{\text{Bulk}}$
D1 Deer	-277 (1.0)	-179 (1.1)	-13.4 (2.6)	-11.2 (1.9)	-31.9 (1.2)	-33.5 (0.7)	-24.9 (0.9)	-20.9 (1.0)	-21.9 (1.2)	-22.8 (1.8)	-19.5 (0.9)	-23.9 (0.6)	-21.7	3.7
D16 Deer	-26.0 (1.1)	-16.0 (2.0)	-23.3 (1.6)	-16.0 (1.2)	-32.7 (0.9)	-32.2 (0.9)	-22.8 (0.8)	-20.4 (0.9)	-22.9 (1.0)	-18.4 (1.8)	-16.4 (1.1)	-22.8 (0.6)	-21.1	4.1
D25 Deer	-24.6 (1.1)	-16.0 (2.4)	-21.2 (0.9)	-16.8 (1.7)	-33.4 (1.3)	-30.4 (0.8)	-22.0 (0.6)	-19.7 (0.6)	-22.0 (0.9)	-18.0 (1.1)	-18.7 (0.8)	-23.1 (0.6)	-21.7	3.6
D3 Dog	-17.6 (1.6)	-5.4 (1.5)	0.5 (1.2)	2.3 (1.2)	-25.4 (2.1)	-25.5 (0.7)	-17.3 (0.8)	-13.1 (0.7)	-11.7 (0.9)	-13.5 (1.1)	-9.3 (0.8)	-18.4 (0.7)	-12.1	16.1
D4 Dog	-15.3 (1.1)	-4.5 (1.8)	-4.0 (1.2)	-2.9 (1.4)	-23.0 (0.9)	-22.0 (0.7)	-20.7 (2.4)	-13.4 (0.6)	-12.4 (0.7)	-10.2 (1.1)	-6.8 (0.8)	-16.4 (0.7)	-12.0	14.7
D6 Dog	-16.9 (1.1)	-4.6 (1.5)	-6.1 (1.1)	-5.5 (2.2)	-25.7 (1.1)	-23.1 (0.8)	-20.3 (1.1)	-14.7 (1.3)	-12.3 (1.5)	-11.6 (1.3)	-6.6 (1.1)	-17.3 (0.7)	-12.7	14.9
D2 Sealion	-16.1 (1.1)	-3.1 (1.5)	-14.3 (1.2)	-5.9 (1.4)	-17.1 (0.9)	-23.2 (0.8)	-15.2 (0.8)	-13.3 (0.9)	-12.0 (0.8)	-11.7 (1.3)	-7.3 (1.1)	-16.6 (0.7)	-12.0	17.7
D38 Salmon	-16.0 (1.1)	-3.3 (1.5)	-24.5 (1.2)	-9.0 (1.4)	-26.7 (0.9)	-25.4 (0.8)	-21.9 (0.8)	-14.9 (0.9)	-17.2 (0.7)	-12.8 (1.1)	-8.7 (1.1)	-19.0 (0.8)	-14.5	11.1
D42 Salmon	-18.1 (0.8)	-3.4 (1.5)	-23.3 (2.1)	-5.2 (1.9)	-27.6 (1.7)	-24.7 (1.6)	-22.5 (0.8)	-15.6 (0.9)	-15.0 (1.3)	-13.4 (1.3)	-7.6 (1.1)	-21.5 (0.7)	-14.0	13.1
D41 Salmon	-16.7 (1.1)	-6.0 (1.5)	-14.5 (1.2)	-7.8 (1.4)	-33.1 (0.9)	-28.2 (0.6)	-18.6 (0.8)	-18.3 (1.0)	-16.1 (1.0)	-14.2 (1.3)	-11.4 (1.1)	-21.6 (0.6)	-15.6	9.5
KDT Bone ^c	-17.5 (1.0)	-4.6 (1.1)	-17.5 (1.2)	-10.2 (2.8)	-14.7 (1.0)	-25.6 (0.6)	-21.7 (0.6)	-12.6 (0.6)	-15.2 (0.9)	-14.6 (1.3)	-11.5 (1.1)	-20.2 (0.6)	-13.8 ^a	17.9 ^a
KDT Skin	-21.4 (1.1)	-9.1 (1.3)	-4.9 (1.2)	-0.2 (1.1)	-30.8 (1.2)	-27.5 (1.1)	-11.0 (1.7)	-15.9 (0.6)	-14.6 (0.7)	-16.4 (1.1)	-14.5 (0.9)	-21.8 (0.6)	-14.8 ^b	9.9 ^b

The numbers in brackets are the errors associated with $\delta^{13}\text{C}$ value determinations.

^a Bone collagen $\delta^{13}\text{C}/\delta^{15}\text{N}_{\text{Bulk}}$ values are for a tibia not the rib from which amino acids were extracted.

^b Skin $\delta^{13}\text{C}/\delta^{15}\text{N}_{\text{Bulk}}$ values are actually muscle $\delta^{13}\text{C}/\delta^{15}\text{N}_{\text{Bulk}}$ values.

^c KDT, the Kwäḏay Dän Ts'incẖ individual.

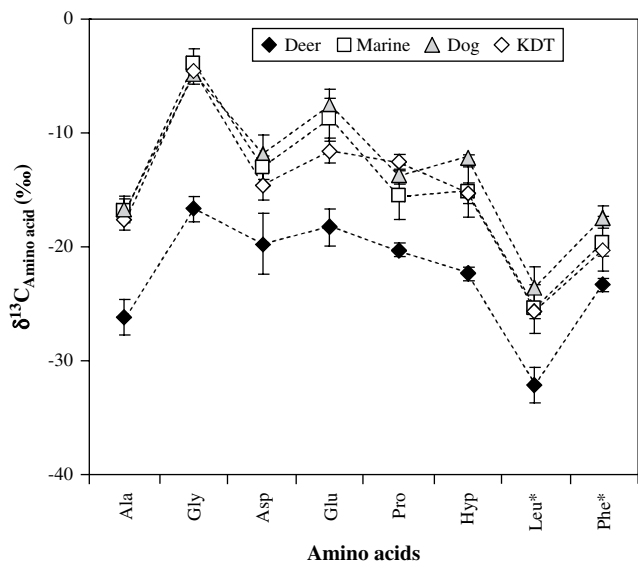


Fig. 3. $\delta^{13}\text{C}$ values of amino acids extracted from bone collagen of the Kwäday Dän Ts'inchj individual (KDT) and faunal species from Dionisio Point in British Columbia. Terrestrial species: deer ($n = 3$), dog ($n = 3$); marine species: salmon, ($n = 3$) and sealion ($n = 1$). * denotes essential amino acid.

$\delta^{13}\text{C}$ values as a palaeodietary analogue for humans, who are only sporadically unearthed in excavations in British Columbia. Although the divergence between the terrestrial and marine animals was highly significant for each amino acid, it was observed, on closer inspection, that the separation is most pronounced for glycine (11.8‰) and least for phenylalanine (2.1‰), a pattern which has been observed previously in humans and faunal species from South Africa (Corr et al., 2005). It was therein demonstrated that marine species and high marine-protein consuming humans exhibited significantly higher $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values (by ca. 8‰) than terrestrial species and terrestrial-food consuming humans. This increase in $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values in marine samples was shown to be associated with an increase in bulk collagen $\delta^{15}\text{N}$ values characteristic of high trophic level activity, such as marine-food consumption. $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values for the Dionisio Point faunal species and the Kwäday Dän Ts'inchj individual's collagen are presented in Fig. 4(a). As anticipated, the terrestrial ($6.6 \pm 0.6\text{‰}$) and marine species ($15.0 \pm 2.1\text{‰}$) significantly differ, by ca. 8.5‰. The Kwäday Dän Ts'inchj individual ($15.6 \pm 1.0\text{‰}$) and the 3 marine-food consuming dogs ($12.5 \pm 0.6\text{‰}$) also have high $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values, the former plotting in the same region as the marine faunal species. A high degree of correlation ($R^2 = 0.78$; $p < 0.001$) was observed between $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ and $\delta^{15}\text{N}_{\text{Bone collagen}}$ values (Fig. 4(b)), confirming the association between highly enriched marine diets and increased $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values. Increased $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values in such marine samples results from anomalously high glycine rather than low phenylalanine $\delta^{13}\text{C}$ values in both marine species/consumers and marine sediments (Fantle et al., 1999; Keil and Fogel, 2001), and in humans is possibly explained by the increased absorption of highly ^{13}C -enriched marine dietary glycine due to the elevated occurrence of this amino acid in marine diets (Corr et al., 2005). This extremely high $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ value seems to confirm the interpretation that the Kwäday Dän Ts'inchj individual spent much of his life as a coastal dweller. Certainly, both ethnographic and environmental evidence for the period 6500 BP to the present attest to the heavy reliance of coastal dwellers on salmonids, nearshore fish, intertidal shellfish, sea mammals and sea birds (Dickson et al., 2004).

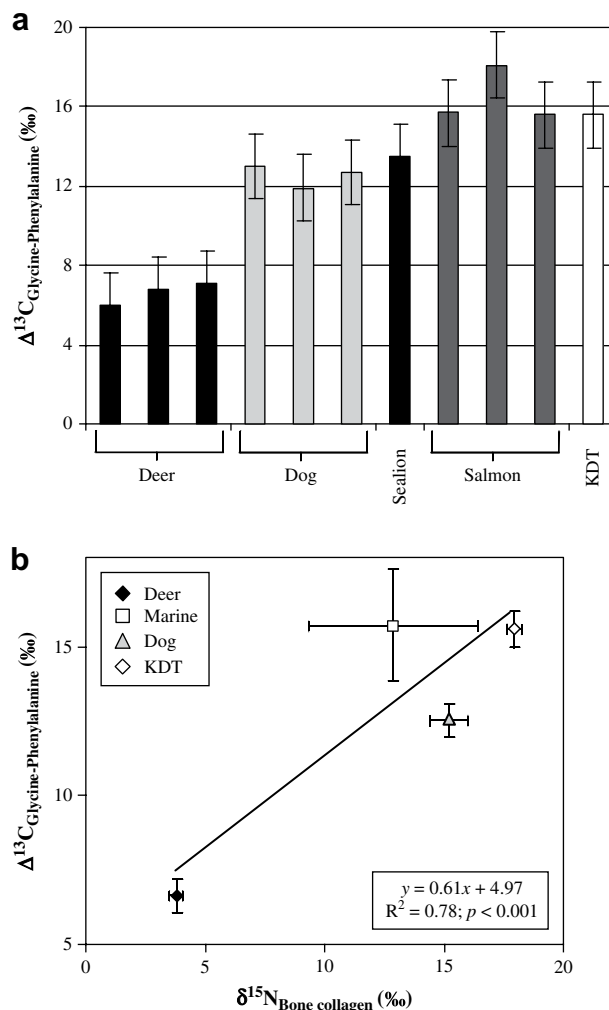


Fig. 4. (a) $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values for the Kwäday Dän Ts'inchj individual (KDT) and marine faunal species from Dionisio Point, (b) relationship between bone collagen $\delta^{15}\text{N}$ and $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values for the Kwäday Dän Ts'inchj individual and faunal species from Dionisio Point in British Columbia. Terrestrial species: deer ($n = 3$), dog ($n = 3$); marine species: salmon, ($n = 3$) and sealion ($n = 1$).

3.4. Comparison of bone and skin collagen amino acid $\delta^{13}\text{C}$ values

In order to detect a possible divergence in the dietary habits of the Kwäday Dän Ts'inchj individual in the last months of life, compound-specific carbon isotope analysis was performed on the Kwäday Dän Ts'inchj individual's skin (rapid turnover; Rucklidge et al., 1992) for comparison with bone amino acid $\delta^{13}\text{C}$ values (slow turnover; Gerber and Altman, 1960). However, in order to enable this comparison we must address potential differences in the carbon isotope composition of amino acids between the two tissues due to metabolic/biosynthetic differences. Since the amino acid compositions of the two tissues were almost identical it can be assumed that the bulk of the skin comprised collagen (see Figs. 1 and 2). There is little experimental demonstration of $\Delta^{13}\text{C}_{\text{Bone collagen-Skin}}$ amino acid values from previous isotopically-controlled animal-feeding experiments; however, Hare et al. (1991) found no significant difference between pig muscle and collagen with respect to glutamate, aspartate, threonine (essential), and serine $\delta^{13}\text{C}$ values.

Fig. 5(a) presents the Kwäday Dän Ts'inchj individual's bone and skin amino acid $\delta^{13}\text{C}$ values, while Fig. 5(b) displays $\Delta^{13}\text{C}_{\text{Bone collagen-Skin}}$ values. Skin amino acid $\delta^{13}\text{C}$ values were observed to track those of bone collagen, indicating the

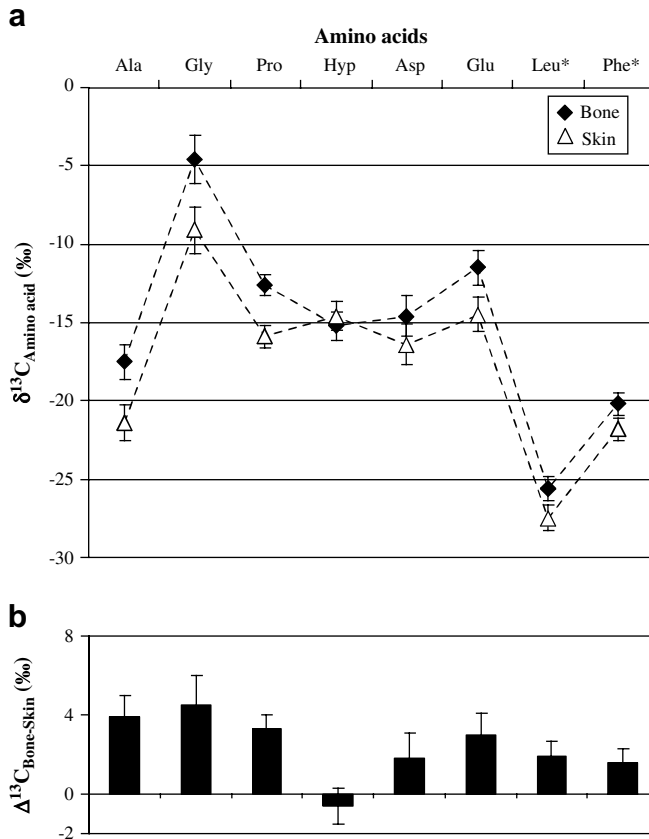


Fig. 5. (a) Comparison of amino acid $\delta^{13}\text{C}$ values for the Kwáday Dän Ts'inchí individual bone and skin, (b) $\Delta^{13}\text{C}_{\text{Bone-Skin}}$ amino acid values. * denotes essential amino acid.

biosynthetic/dietary origin of collagen carbon in both tissues to be analogous. With the exception of hydroxyproline all skin collagen amino acids were lower than those of bone collagen by an average $2.4 \pm 1.6\text{‰}$, ranging from 1.6‰ for phenylalanine to 4.5‰ for glycine. This sharp decrease in the tissue glycine $\delta^{13}\text{C}$ value compared to other amino acids in the last months prior to death undoubtedly results from a decrease in marine food consumption. No significant differences are observed between marine and C_3 terrestrial environment phenylalanine $\delta^{13}\text{C}$ values (Keil and Fogel, 2001); hence, a decrease by only 1.6‰ in combination with a more substantial decrease in glycine $\delta^{13}\text{C}$ values is likely to reflect a divergence away from marine dietary sources. This results in a reduction of $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values from $15.6 \pm 0.7\text{‰}$ for bone to $12.7 \pm 0.9\text{‰}$ for skin. The latter is intermediate between those values observed for marine and terrestrial consumers in the present investigation and a previous study of the dietary preferences of South African coastal hunter-gatherers (Corr et al., 2005) and likely reflects a combination of an 'old' ^{13}C -enriched collagen glycine pool and a recent more ^{13}C -depleted pool of collagen glycine *de novo* synthesised from dietary carbohydrates from C_3 terrestrial sources. Interestingly, the non-essential amino acids alanine, glycine, proline and glutamate show a higher degree of ^{13}C -enrichment between bone and skin than the essential amino acids, leucine and phenylalanine. While the latter two essential amino acids are similarly ^{13}C -depleted in marine and terrestrial animals, the former non-essential amino acids are more ^{13}C -enriched in marine animals and sediments (Corr et al., 2005; Keil and Fogel, 2001), thus providing further evidence for a shift towards a terrestrial diet in the months prior to death.

There is strong evidence that the shift in bone to skin individual amino acid $\delta^{13}\text{C}$ values represents the signature of a coastal dweller

who embarked on a single journey rather than a seasonal excursion inland. Certainly, the high bone collagen amino acid $\delta^{13}\text{C}$ values and $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ value of $15.6 \pm 1.0\text{‰}$ do not provide for a meaningful C_3 terrestrial dietary intake throughout life. The possibility that the 'marine' dietary signal evident in the Kwáday Dän Ts'inchí individual's bone collagen amino acids derives from the consumption of Pacific salmon during their inland migrational spawning season up the Tatshenshini River and that he had not been a coastal dweller, is unlikely because of the magnitude of the ^{13}C -enrichment observed in bulk collagen and $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values, which do little to suggest even a hint of the other terrestrial foods which would have been abundantly available inland. Furthermore, the inland availability of migratory Pacific salmon is restricted to the spawning season with a 6-month duration from August to January (Szepanski et al., 1999). Salmon were preserved in quantity for use during all times of the year, but reliance on these fish would lessen when there were no runs. Therefore, there were at least a few months per annum of increased human reliance on ^{13}C -depleted terrestrial food sources. Hence, the minimum reliance on terrestrial relative to marine dietary sources of an inland dweller would likely approximate 50% of dietary intake which is not at all consistent with the extremely high amino acid $\delta^{13}\text{C}$ values observed for this individual. Thus, the results of both bulk and compound-specific isotope analysis provide evidence that this individual was a coastal dweller with a considerable reliance on marine dietary sources.

There is insufficient information on skin turnover to establish the extent to which a seasonal dietary shift would impact skin amino acids; however, even a very short annual period inhabited inland would impact bone collagen $\delta^{13}\text{C}$ values, which was not observed in the Kwáday Dän Ts'inchí individual. Hence, the necessary conclusion is that the divergence between bone and skin amino acid $\delta^{13}\text{C}$ values and $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values reflects a single journey inland where he spent the final months of life. The lower skin amino acid $\delta^{13}\text{C}$ values are likely to represent the introduction of herbivorous protein from animals indigenous to this region of British Columbia, such as deer, caribou, sheep and mouse (Beattie et al., 2000; Cannon et al., 1999; Lovell et al., 1986). The possibility of freshwater rather than, or in addition to, terrestrial food sources in the last months of life cannot be discounted. However, the consumption of migratory salmon from freshwater sources during the last months of life can be discounted. Salmon cease feeding during the spawning season; hence, there is no associated freshwater isotope signal for this period; indeed, the resulting nutritional stress has been shown to result in a slight ^{13}C -enrichment in red muscle and liver tissues (ca. $2\text{--}4\text{‰}$; Doucett et al., 1999). Freshwater and terrestrial food sources have very similar $\delta^{13}\text{C}$ values, although it is not yet possible to confirm whether they are distinguishable with respect to $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values. High $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values in humans are likely to result from the direct incorporation of ^{13}C -enriched glycine from fish/marine mammals; while it seems that ^{13}C -enrichment in glycine accumulates through successive trophic levels (Fantle et al., 1999), and thus would be anticipated in freshwater high trophic level consumers. The dynamics in $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values in humans with a substantial freshwater fish component to their diet requires investigation.

4. Conclusions

We have demonstrated herein the utility of compound-specific carbon isotope analysis of the amino acids of human tissues to probe dietary shifts related to migration through contrasting geographical areas. Accordingly, observed dietary isotopic shifts between tissues of different turnover rates were shown to be constructive in identifying the geographical history of the Kwáday

Dän Ts'inchj individual. The comparison of 8 of the Kwäday Dän Ts'inchj individual's bone and skin amino acids and, more specifically, $\Delta^{13}\text{C}_{\text{Glycine-Phenylalanine}}$ values, indicated a shift from a long-term average diet dominated by marine foods (bone signal) followed by a shift towards terrestrial/freshwater resources in the last months of life (skin signal). Application of the compound-specific stable isotope technique confirmed that the Kwäday Dän Ts'inchj individual was inland long enough for the inland terrestrial/freshwater diet to register in his skin collagen amino acids. However, the lack of a terrestrial signal in bone collagen amino acids supports the hypothesis that this was a single or very occasional journey of the Kwäday Dän Ts'inchj individual, a coastal dweller into the British Columbian interior.

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References

- Beattie, O., Aplaud, A., Blake, E.W., Cosgrove, J.A., Gaunt, S., Greer, S., Mackie, A.P., Mackie, K.E., Straathof, D., Thorp, V., Troffe, P.M., 2000. The Kwaday Dan Ts'inchj discovery from a glacier in British Columbia. *Canadian Journal of Archaeology* 24, 129–147.
- Cannon, A., Schwarcz, H.P., Knyf, M., 1999. Marine-based subsistence trends and the stable isotope analysis of dog bones from Namu, British Columbia. *Journal of Archaeological Science* 26, 399–407.
- Cerling, T.E., Ehleringer, J.R., West, A., Stange, E., Dorigan, J., 2003. Forensic applications of stable isotopes in hair. *Forensic Science International* 136, 172–172.
- Chisholm, B.S., Nelson, D.E., Schwarcz, H.P., 1982. Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. *Science* 216, 1131–1132.
- Chisholm, B.S., Nelson, D.E., Schwarcz, H.P., 1983. Marine and terrestrial protein in prehistoric diets on the British Columbia coast. *Current Anthropology* 24, 396–398.
- Corr, L.T., Sealy, J.C., Horton, M.C., Evershed, R.P., 2005. A novel marine dietary indicator utilising compound-specific bone collagen amino acid $\delta^{13}\text{C}$ values of ancient humans. *Journal of Archaeological Science* 32, 321–330.
- Corr, L.T., Richards, M.P., Jim, S., Ambrose, S.H., Mackie, A., Evershed, R.P., 2008. Probing dietary change of the Kwäday Dän Ts'inchj individual, an ancient glacier body from British Columbia: I. Complementary use of marine lipid biomarker and carbon isotope signatures as novel indicators of a marine diet. *Journal of Archaeological Science* 35, 2102–2110.
- Csapo, J., CsapoKiss, Z., Wagner, L., Talos, T., Martin, T.G., Folestad, S., Tivesten, A., Nemethy, S., 1997. Hydrolysis of proteins performed at high temperatures and for short times with reduced racemization, in order to determine the enantiomers of D- and L-amino acids. *Analytica Chimica Acta* 339, 99–107.
- DeNiro, M.J., 1987. Stable isotope and archaeology. *American Scientist* 75, 182–191.
- Dickson, J.H., Richards, M.P., Hebda, R.J., Mudie, P.J., Beattie, O., Ramsay, S., Turner, N.J., Leighton, B.J., Webster, J.M., Hobischak, N.R., Anderson, G.S., Troffe, P.M., Wigen, R.J., 2004. Kwaday Dan Ts'inchj, the first ancient body of a man from a North American glacier: reconstructing his last days by intestinal and biomolecular analyses. *Holocene* 14, 481–486.
- Docherty, G., Jones, V., Evershed, R.P., 2001. Practical and theoretical considerations in the gas chromatography/combustion/isotope ratio mass spectrometry $\delta^{13}\text{C}$ analysis of small polyfunctional compounds. *Rapid Communications in Mass Spectrometry* 15, 730–738.
- Doucett, R.R., Booth, R.K., Power, G., McKinley, R.S., 1999. Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (*Salmo salar*): insights from stable-isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 2172–2180.
- Ehleringer, J.R., 2005. Stable isotopes in forensic chemistry. *Abstracts of Papers of the American Chemical Society* 229, U1182. –U1182.
- Elster, H., Gilav, E., Weiner, S., 1991. Amino acid racemization of fossil bone. *Journal of Archaeological Science* 18, 605–617.
- Fantle, M.S., Dittel, A.I., Schwalm, S.M., Epifanio, C.E., Fogel, M.L., 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. *Oecologia* 120, 416–426.
- Fraser, I., Meier-Augenstein, W., Kalin, R.M., 2006. The role of stable isotopes in human identification: a longitudinal study into the variability of isotopic signals in human hair and nails. *Rapid Communication in Mass Spectrometry* 20, 1109–1116.
- Gerber, G., Altman, K.I., 1960. Studies on the metabolism of tissue proteins. 1. Turnover of collagen labelled with proline- U-C^{14} in young rats. *Journal of Biological Chemistry* 235, 2653–2656.
- Hare, P.E., Fogel, M.L., Stafford, T.W., Mitchell, A.D., Hoering, T.C., 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *Journal of Archaeological Science* 18, 277–292.
- Herring, G.M., 1972. *The Organic Matrix of Bone*. Academic Press New York.
- Howland, M.R., Corr, L.T., Young, M.M., Jones, V., Jim, S., van der Merwe, N.J., Mitchell, A.D., Evershed, R.P., 2003. Expression of the dietary isotope signal in the compound-specific $\delta^{13}\text{C}$ values of pig bone lipids and amino acids. *International Journal of Osteoarchaeology* 13, 54–65.
- Jim, S., Jones, V., Copley, M.S., Ambrose, S.H., Evershed, R.P., 2003. Effects of hydrolysis on the $\delta^{13}\text{C}$ values of individual amino acids derived from polypeptides and proteins. *Rapid Communications in Mass Spectrometry* 17, 2283–2289.
- Jones, V., 2002. Investigating the Routing and Synthesis of Amino Acids between Diet and Bone Collagen via Feeding Experiments and Applications to Palaeodietary Reconstruction. School of Chemistry, University of Bristol, UK.
- Keil, R.G., Fogel, M.L., 2001. Reworking of amino acid in marine sediments: stable carbon isotopic composition of amino acids in sediments along the Washington coast. *Limnology and Oceanography* 46, 14–23.
- Koch, P.L., Fogel, M.L., Tuross, N., 1994. Tracing the diets of fossil animals using stable isotopes. In: Lajtha, K., Michener, R.H. (Eds.), *Methods in Ecology, Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific, Oxford, pp. 63–92.
- Lovell, N.C., Chisholm, B.S., Nelson, D.E., Schwarcz, H.P., 1986. Prehistoric salmon consumption in interior British Columbia. *Canadian Journal of Archaeology* 10, 99–106.
- Madigan, M.T., 2003. Cell structure/function. In: Madigan, M.T. (Ed.), *Brock Biology of Microorganisms*, Chapter 4. Pearson Higher Education, New Jersey.
- van der Merwe, N.J., 1982. Carbon isotopes, photosynthesis and archaeology. *American Scientist* 70, 596–606.
- van der Merwe, N.J., Vogel, J.C., 1978. ^{13}C content of human collagen as a measure of prehistoric diet in woodland North America. *Nature* 276, 815–816.
- Monsalve, M.V., Stone, A.C., Lewis, C.M., Rempel, A., Richards, M., Straathof, D., Devine, D.V., 2002. Brief communication: molecular analysis of the Kwaday Dan Ts'inchj ancient remains found in a glacier in Canada. *American Journal of Physical Anthropology* 119, 288–291.
- Mudie, P.J., Greer, S., Brakel, J., Dickson, J.H., Schinkel, C., Peterson-Welsh, R., Stevens, M., Turner, N.J., Shadow, M., Washington, R., 2005. Forensic palynology and ethnobotany of *Salicornia* species (Chenopodiaceae) in northwest Canada and Alaska. *Canadian Journal of Botany* 83, 111–123.
- Price, T.D., 1989. Bones, chemistry, and the human past. In: Price, T.D. (Ed.), *The Chemistry of Prehistoric Human Bone*. Cambridge University Press, Cambridge, pp. 1–9.
- Pringle, H., 2002. Out of the ice: who was the ancient traveller discovered in a Tatshenshini glacier? *Canadian Geographic* 122, 56–64.
- Richards, M.P., Greer, S., Corr, L.T., Beattie, O., Mackie, A., Evershed, R.P., von Finster, A., Southon, J., 2007. Radiocarbon dating and dietary stable isotope analysis of Kwaday Dan Ts'inchj. *American Antiquity* 72, 719–733.
- Roy, D.M., Hall, R., Mix, A.C., Bonnichsen, R., 2005. Using stable isotope analysis to obtain dietary profiles from old hair: A case study from Plains Indians. *American Journal of Physical Anthropology* 128, 444–452.
- Rucklidge, G.J., Milne, G., McGaw, B.A., Milne, E., Robins, S.P., 1992. Turnover rates of different collagen types measured by isotope ratio mass-spectrometry. *Biochimica et Biophysica Acta* 1156, 57–61.
- Schoeninger, M.J., 1989. Reconstructing prehistoric human diet. In: Price, T.D. (Ed.), *The Chemistry of Prehistoric Human Bone*. Cambridge University Press, Cambridge, pp. 38–67.
- Schwarcz, H.P., 2001. Some biochemical aspects of carbon isotopic palaeodiet studies of mice and people. In: Ambrose, S.A., Katzenberg, M.A. (Eds.), *Advances in Archaeological and Museum Science*. Plenum Press, New York, pp. 189–210.
- Silfer, J.A., Engel, M.H., Macko, S.A., Jumeau, E.J., 1991. Stable carbon isotope analysis of amino-acid enantiomers by conventional isotope ratio mass-spectrometry and combined gas-chromatography isotope ratio mass-spectrometry. *Analytical Chemistry* 63, 370–374.
- Szepanski, M.M., Ben-David, M., Van Ballenberghe, V., 1999. Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. *Oecologia* 120, 327–335.
- Tuross, N., Fogel, M.L., Hare, P.E., 1988. Variability in the preservation of the isotopic composition of collagen from fossil bone. *Geochimica et Cosmochimica Acta* 52, 929–935.