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^{14}C MEASUREMENTS ELUCIDATE ISOTOPIC DIFFERENCES BETWEEN NAILS AND HAIR IN MODERN HUMANS

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ABSTRACT. In forensic sciences, radiocarbon found in modern human nails and hair is evaluated to determine the year of death. However, ^{14}C analyses presented herein of fingernails and hair from the same infant demonstrated ^{14}C values of hair that were lower than would be expected (e.g. depleted relative to the fingernails by at least 10‰). These results prompted a series of ^{14}C measurements on infant hair strands, fingernails, and infant shampoo, which suggested the presence of C contamination due to cosmetic products. To further evaluate these discrepancies, several hair strands and fingernail samples from multiple donors were collected, pretreated by several approaches, and measured using isotopic analysis (δ^{13}C, δ^{15}N, and C/N as well as ^{14}C accelerator mass spectrometry). SEM images of the surface of chemically pretreated hair strands were also taken to inspect the performance of the chemical pretreatments applied. Our ^{14}C and stable isotope results show that modern human hair is likely contaminated with fossil-fuel-derived carbon, which is found in most hair care products. Currently, the various chemical pretreatments available in the literature and presented herein show that it is not possible to completely remove foreign carbon contaminates. Thus, the human ^{14}C and δ^{13}C values between keratinous tissues (fingernails and hair) are not in agreement. From these observations, it becomes apparent that isotopic interpretations using human hair should be used with extreme caution and additional work is needed for its use in forensic and dietary research.

INTRODUCTION

Radiocarbon measurements from fast turnover tissues have been used by forensic scientists to precisely determine the year of death using the high concentration of ^{14}C in Earth's atmosphere as a result of thermonuclear weapons testing carried out between 1953 and 1963 (Hodgins 2009; Lynnerup et al. 2010). As ^{14}C is incorporated into the food chain through photosynthesis, its concentration can be measured and used to determine the time of tissue formation. Fingernails and hair may be suited for ^{14}C and isotopic measurements since both tissues linearly and permanently record variations through time (Hodgins 2009). Since hair and fingernails are composed of keratin with nearly identical amino acid profiles (Langbein et al. 1999), bulk protein isotopic values from both tissues in a single individual should in principle yield similar ^{14}C measurements and stable isotopic values. O'Connell et al. (2001) compared isotopic values (δ^{13}C and δ^{15}N) of fingernails and hair from 12 individuals, and found that hair was ^{13}C enriched by 0.2 ± 0.4‰ and ^{15}N depleted by 0.6 ± 0.2‰ when compared to fingernail keratin.

A recent longitudinal study of fingernails and hair from the same infant, collected from 2002–2004 (Figure 1), were measured using ^{14}C accelerator mass spectrometry (AMS). The ^{14}C values of the infant's hair were depleted by 10‰ relative to the fingernails. To investigate this unexpected discrepancy, Santos et al. (2011) performed isotopic measurements on hair and fingernail tissues from different individuals and a commercially available infant/toddler shampoo to determine a potential source of carbon contamination. The shampoo yielded a bulk ^{14}C age of ~8.8 kyr BP and an average δ^{13}C value of –29.2‰ (n = 2). The hair was ^{14}C depleted by 8‰, with larger offsets for "older" hair located farther from the roots. Similarly, Hodgins (2009) had also reported ^{14}C-depleted values while studying hair samples from deceased females with a known year of death. Since their diets were unknown, except for isotopic results, a dietary reservoir effect could not be excluded as the cause of all or a substantial part of the observed ^{14}C differences. Depending on where the ^{14}C result is located along the bomb curve, a ^{14}C offset of 10‰ or more can cause an erroneous 2 to 3 yr calculation of the precise year of death (Hua et al. 2013). Such an erroneous determination of the precise year of death could lead the forensic investigation in the wrong direction or not provide any relevant information on an individual's recent past (Wild et al. 2000; Lynnerup et al. 2010).

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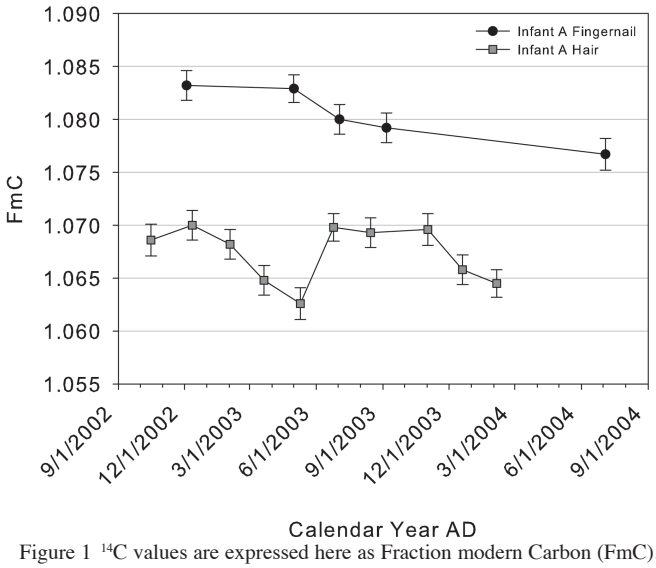

Figure 1 ^{14}C values are expressed here as Fraction modern Carbon (FmC). Data presented are from a single infant (Santos et al. 2011, unpublished data). Samples were acquired from an archived work on stable isotope analyzes of carbon and nitrogen of human infants during breastfeeding and weaning (Fuller et al. 2004, 2005).

The objectives of this paper are threefold. The first is to provide some background information on how the hair structure can be modified by hair care products, which contain petrochemicals and botanical and animal extracts. The second is to demonstrate that these substances can introduce contaminates into the hair and bias isotopic results. For this purpose, longitudinal hair samples collected from a small group of individuals were measured by ^{14}C AMS coupled with stable isotope results and quality control indicators (%C, %N, and C/N). Fingernails from the same subjects were also processed and measured to serve as a reference point to the hair. To assure that the contamination is indeed from petrochemical derivatives, rat hair was also evaluated. Third, in order to determine whether hair subjected to permanent dye coloring can be cleaned of exogenous carbon (C) contaminants that are incorporated into the keratin structure, various chemical treatments were evaluated. Scanning electron microscope (SEM) images were used to evaluate how the chemical cleaning processes tested affected the hair structure, and the treatment's ability to remove some of the contaminated layers. Here, we discuss the implications of C-contaminated hair for the fields of forensics and dietary reconstruction.

KERATINOUS TISSUE AND COSMETIC HAIR CARE PRODUCTS

Fingernails and hair are composed of α-keratin, an extremely resilient protein that is insoluble under natural conditions. Although considered members of the keratin family, fingernails and hair are subtly different in their amino acid composition (Odland and Goldsmith 1991; Langbein et al. 1999). Weathering processes such as abrasion from washing, brushing, and exposure to UV light and heat can strip the hair of its protective outer layer (cuticle) and allow foreign substances to become trapped within the hair matrix (Tsanaclis and Wicks 2008; Stenström et al. 2010; Auerswald et al. 2011). Hair also undergoes various cosmetic treatments that alter the chemical composition of the keratin structure. Hair dyeing, bleaching, shampooing, and conditioning can cause the cuticle to open up and expose the inner layers of the hair shaft to contamination (Wennig 2000; Clausen et al. 2006; Wilson et al. 2010). Hair strands can also become contaminated before emerging from the hair follicle. Cosmetic hair care products are designed to penetrate and treat the scalp (Oshimura et

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al. 2007), allowing an opportunity for contaminates to incorporate themselves into freshly formed hair. Cosmetic hair care products contain several ingredients that vary from brand to brand, but in general all share a set of chemicals that are universal (Hunting 1983, 1987). A significant number of these chemicals are petroleum based. Some of the known petrochemical derivatives are propylene/butylene glycol, petrolatum, liquid paraffin or mineral oil, color additives or dyes, and surfactants to name just a few (Evans and Wickett 2012). Hair care products also contain substances that are extracted from plants and animals or are synthesized using petroleum to mimic naturally occurring compounds (Hunting 1983, 1987). These compounds are known to facilitate and enhance the penetration of chemical reagents beyond the cuticle layer (Mainkar and Jolly 2001; Kuzuhara and Hori 2003; Oshimura et al. 2007; Feigenbaum 2009; Evans and Wickett 2012).

Similar to hair, fingernails grow at various rates that are dependent on the health, gender, and age of an individual. Fingernails record an individual's dietary habits as cells are expelled from the skin forming the nail plate that continues to grow until it extends beyond the fingertip (Hopps 1977; Zaias 1990; Hodgins 2009). Fingernails are also subjected to weathering processes and to contamination such as cosmetic treatments and medications that can open up micropores on the nail plate and introduce foreign substances (Hao and Li 2008).

MATERIALS AND METHODS

Subjects and Sample Collection

Fingernail and hair samples from humans were collected from a group of 4 healthy volunteers (Table 1). In addition, a control group of 3 adult rats raised using a commercial rodent diet were studied. Subjects were selected due to their particular grooming habits and general diet. These individuals were from different ethnic backgrounds, and demonstrated some level of loyalty towards their original food traditions. Since they were living in the same region (southern California) at the time of sample collection, they were all subjected to today's globalized food supply (Nardoto et al. 2006; Von Braun and Diaz-Bonilla 2008; Hodgins 2009). After explaining the purpose of the study, all volunteers or their guardians gave informed consent for participation. Participants' hair was collected and cut every 1 cm from the root to the tip.

Fingernails were collected to serve as references (analyzed in bulk) to the hair of each individual. Since nail growth rates range from 1.5 to 3 mm per month, it takes about 4 to 6 months for the nail cuticle to reach the fingertip before sampling (Lehn et al. 2011). In order to match fingernails to the timeframe of hair growth (in cm), we used the average growth rates of fingernails and hair provided in the literature (Lehn et al. 2011) and the date of collection. In addition, a control group of 3 adult rats raised using a commercial rodent diet was studied. The fingernails and hair of the control group were not exposed to cosmetic products and serve as an independent reference to evaluate if C contamination was the source of the nail-hair offsets observed previously (Santos et al. 2011).

Sample Chemical Pretreatments

In order to remove contaminates prior to analysis, a modified chemical pretreatment known as acid-base-acid (ABA; Santos and Ormsby 2013) was selected as a reference for comparison to other chemical pretreatments applied in this study. The alkaline portion of the ABA pretreatment was carried out at room temperature to avoid keratin degradation and loss (Clausen et al. 2006). Samples were then washed with 18.2MΩ DDH_2O water until pH neutral, and dried in a vacuum oven with no heat. The ABA pretreatment was used on all hair samples, except those that underwent specialized chemical pretreatment regiments, as indicated in Table 2.

Several pretreatments to remove hair dye as well as other cosmetic products were developed for

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human hair (Table 2). A procedure using a commercial hair dye remover for 60 min coupled with detergent (Conrad®) washes was tested. The detergent was used to remove lipids and to strip off the hair scales (as suggested by Taylor et al. 1995). Following this specialized treatment, an ABA treatment (as mentioned above) was applied to remove detergent residue (if any), and for additional cleansing of hair samples prior to isotopic analysis. A chemical pretreatment using sequential (6–10) hydrogen peroxide (3% H_2O_2) baths at 60°C was used due to its ability to break apart hair dye pigment compounds (Clausen et al. 2006). We applied this treatment for ~30 min until the hair appeared colorless, indicating that the breakdown of the melanin pigments within the hair strands and dye had reached completion. A combination of commercial hair dye remover and hydrogen peroxide (3% H_2O_2) was also examined without the addition of the ABA procedure (Table 2). Once the chemical pretreatments were completed, samples were washed with 18.2MΩ DDH_2O water until pH neutral and dried in a vacuum oven with no heat.

Table 1 Subjects' general information, such as age, gender, diet, general grooming habits, and the rationale used for inclusion in this study are indicated. Adult rats were selected as controls as their keratinous tissues (analyzed in bulk vs. subsectioned) were free of cosmetic products.

Subject	Age (yr)	Gender	Diet	Grooming habits	Rationale
Human (A)	20	Female	90% terrestrial (mostly grains, vegetables, fruits and nuts) and 10% marine	Painted nails, hair washing†, hair styling treatment‡	Subject uses typical cosmetic treatments for her age group
Human (B)	45	Female	90% terrestrial (mostly fruits, vegetables, and dairy products, followed by meat and eggs) and 10% marine	Hair washing†, hair dying^	Subject uses extremely aggressive cosmetic treatments to cover gray hair
Human (C)	3	Male	~95% terrestrial (mostly fruits, vegetables, and meat followed by eggs and dairy products) and <5% marine	Hair washing†	Subject uses mild cosmetic products for his age group
Human (D)	23	Male	90% terrestrial (mostly meat and corn) and 10% marine	Hair washing†; hair styling treatment‡	Subject makes mild use of cosmetic products
Rat (1)	>1*	Male	Rat feed	Self-groomer	Control (no cosmetic products)
Rat (2)	>1*	Male	Rat feed	Self-groomer	Control (no cosmetic products)
Rat (3)	>1*	Male	Rat feed	Self-groomer	Control (no cosmetic products)

†Hair washing = cleaning of hair with shampoo and conditioner. ‡Hair styling treatment = application of chemical products to hair. ^Hair dying = use of dying agents to alter hair color. *At this age, most male rats are fully grown and developed.

The fingernail samples from all subjects were polished using sandpaper to remove surface contaminates; however, subject (A) required nail polish remover before this procedure could be applied. Once collected, samples were cryogenically crushed into <3-mm fragments. To avoid variability due to fingernail growth, each fingernail type (thumb and digits) was homogenized before analyses. Fingernail fragments were sonicated in acetone for 60 min followed by ethanol for an additional

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60 min to remove contaminates. Several 18.2MΩ DDH_2O water washes were applied for 10 min until samples solutions were pH neutral. This protocol was adapted from the cleaning procedure of O'Connell et al. (2001). Once pretreated, the samples were dried using a vacuum oven without heat.

Table 2 Specialized chemical pretreatments developed to remove cosmetic dye from hair.

Treatment	Hair dye removal	Time (min)	Scale removal	Time (min)	Foreign carbon removal	Time (min)
1	L'Oreal® dye remover	60	Conrad® detergent	30	Acid-base-acid	30–60–30
2	L'Oreal dye remover	60	Conrad detergent	30	(Acid-base-acid) ×2	30–60–30
3	L'Oreal dye remover - Hydrogen peroxide (3%)	60–30	N/A	N/A	N/A	N/A
4	Hydrogen peroxide (3%)	30	N/A	N/A	N/A	N/A

ANALYTICAL ANALYSES

Radiocarbon Analysis

For ^{14}C AMS measurements, CO_2 was produced by combustion using approximately 1.6 mg of pretreated material loaded into prebaked quartz tubes with Ag wire and CuO. The tubes were then evacuated of atmospheric gases, sealed, and baked at 900°C/3 hr. The CO_2 evolved was cryogenically cleaned and reduced to graphite in the presence of H_2 at 550°C over an iron powder catalyst (Santos et al. 2007). Graphite samples were measured using a compact AMS system (NEC 0.5MV 1.5SDH-1) with a typical precision of 2–3‰ (Beverly et al. 2010). ^{14}C results were normalized to a set of 6 independent targets of oxalic acid I (primary standard). For quality control, samples were followed by ^{14}C-free samples (USGS coal) and secondary standards of organic nature (FIRI-G barley) prepared in the same manner as the unknowns. We chose to report the ^{14}C results as FmC (Fraction modern Carbon, equivalent to the $F^{14}C$ as advocated in Reimer et al. 2004) in Table S1 (online Supplementary file) and Figures 2 and 6 to facilitate the direct comparisons of tissues ^{14}C results with bomb-^{14}C records. Hair results are reported with respect to the length from root to tip (left to right on the *x* axis, Figure 2). However, many of these "root-hair" samples were lost during chemical pretreatments or did not produce enough CO_2 for an accurate measurement.

Stable Isotope Analysis

Stable isotope results are measured as the ratio of the heavier isotope to the lighter isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and reported as δ values in parts per 1000 or per mil (‰) relative to internationally defined standards for carbon (Vienna Pee Dee Belemnite, VPDB) and nitrogen (ambient inhalable reservoir, AIR) (Schwarcz and Schoeninger 1991). Here, aliquots of chemically clean fingernail fragments and hair ranging from 0.6 to 0.7 mg each were weighed into tin containers. For comparison, we also performed stable isotope measurements on some of the bulk cosmetic hair products used by our volunteers. Samples were then combusted to CO_2 and N_2 in an automated carbon and nitrogen mass spectrometer (Finnigan Delta-Plus CF-IRMS) interfaced with a Fisons NA 1500NC elemental analyzer (EA). This instrument has a typical precision of 0.1‰ based on measurements of several in-house reference materials (atropine, cysteine, lysine, and tryptophan), which are constantly tested and calibrated against standard gas mixtures.

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SEM Imaging

Scanning electron microscope (SEM) images were taken along longitudinal sections of hair strands that were subjected to specialized chemical pretreatments (Table 2). Before imaging, the hair strands were mounted on double-sided carbon tape adhered to aluminum stubs, and made conductive by coating with gold/palladium (VG/Polaron SC 7620 sputter coater). Samples were then loaded into a Schottky thermal field emission FEI/Philips XL-30 SEM with back-scattered electron detector. Images were obtained at various magnifications.

RESULTS AND DISCUSSION

Human Radiocarbon Analysis

Figure 2(a–d) shows all ^{14}C results from the human subjects tested, and the hair results (1.0232 to 1.0385 FmC) show a significant scatter, that if alone could be attributed to dietary preferences. However, the hair samples are clearly ^{14}C depleted (~5–13‰) when compared to the fingernail references. Furthermore, the scatter from the fingernail ^{14}C results (1.0408 to 1.0445 FmC) is much smaller, regardless of the fact that the subjects studied here are from different age groups, genders, and nutritional profiles (Table 1). A marine diet reservoir effect could cause shifts in the expected ages, as observed by Arneborg et al. (1999). However, this explanation is not valid in this case, as a marine diet reservoir effect should influence all the tissues and not just hair. Santos et al. (2011) previously showed that even an infant/toddler hair shampoo can contain small amounts of fossil-fuel-

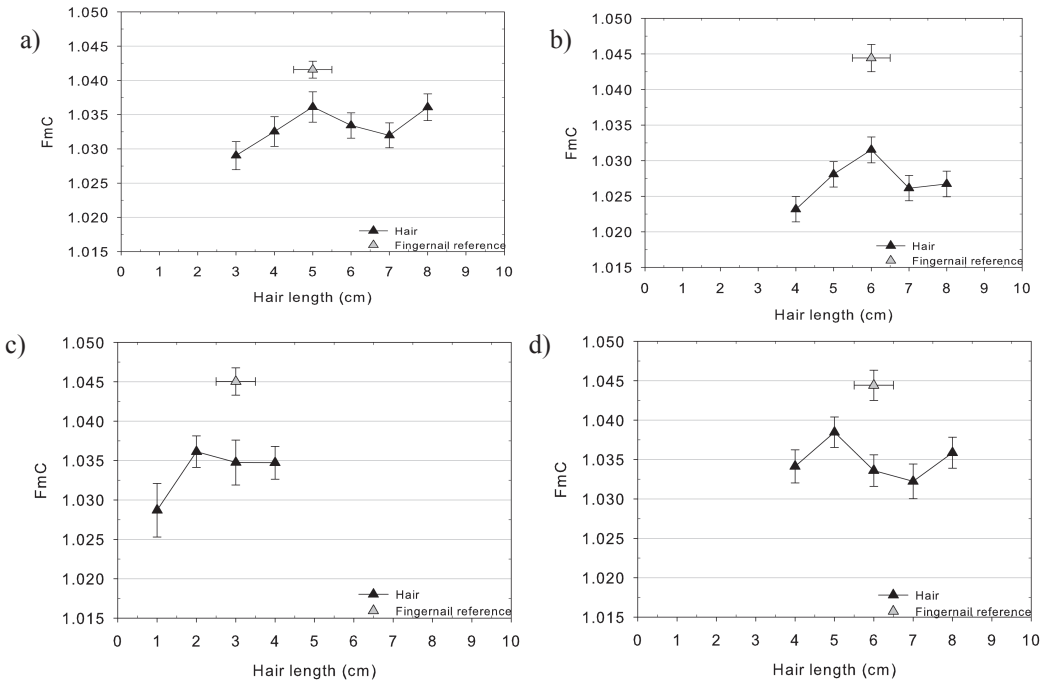

Figure 2 (a–d) Radiocarbon results of all human subjects are reported in FmC. Hair length is measured from the root to the tip (left to right). Nail results represent an average growth of 1 to 1.5 months and are provided for comparison. We estimated an error of 2 weeks (equivalent to 0.5 cm of hair growth) to the timeframe of the fingernails. The fingernail position was aligned to the timeframe represented in relation to the hair. All subjects' hair values are ^{14}C depleted with respect to the nail. The nail-hair offsets for each subject are (A) = 5.4‰, (B) = 12.9‰, (C) = 6.0‰, and (D) = 10.8‰.

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derived carbon (FmC = 0.3331 ± 0.0009; n = 2; equivalent to ~8.8 kyr BP). Although we have not ^{14}C measured a large array of hair care products used by the subjects in this study, our hair ^{14}C results support the argument that carbon contaminants found in some shampoos, conditioners, and treatment products can be incorporated within the hair structure, biasing the ^{14}C values. Moreover, it appears that this contamination is nonsystematic towards modern or depleted values. This is not unexpected, as hair care products contain a wide assortment of naturally occurring and petroleum-based synthesized ingredients (Hunting 1983, 1987; Evans and Wickett 2012). In addition, our human subjects did not use just one cosmetic product during the lifetime (which consists of continuous growth of hair prior to shedding; Bergfeld and Mullinari-Brenner 2001) of their hair strands prior to collection. Without a continuous use of a single cosmetic product, any apparent patterns of sequential decreasing or increasing of ^{14}C values in hair could not to be detected.

On the other hand, the nail-hair offset is most noticeable in subject (B), who regularly used dark permanent hair coloring (known to contain petroleum-based dye; see the large nail-hair offset of 12.9‰ in Figure 2b). Subject (A) uses nail polish (also a known fossil-fuel-derived cosmetic product). This contamination is not apparent in the ^{14}C results, possibly due to its superficial application, which can be removed before sampling. Nail polish remover was used [subject (A) only] in addition to fine sandpaper to remove the top layer of the nail plate *in situ*, before performing chemical pretreatment.

Stable Isotope Analysis of Humans and Hair Care Products

Stable isotope ratios are important as a way to track variations in primary source materials and dietary reconstruction (Meier-Augenstein 2011). The $\delta^{13}C$ values can be used to estimate the flow of carbon in the food chain from diet to subject tissue, while $\delta^{15}N$ is used to estimate trophic level position (Schwarcz and Schoeninger 1991). Tissue protein stable isotope values can be checked for contamination and degradation using quality control indicators such as %C, %N, and C/N (van Klinken and Hedges 1995; Contrino et al. 1996). For well-preserved bone collagen, an atomic C/N ratio of 2.9–3.6 was established by DeNiro (1985). However, for fingernails and hair, O'Connell et al. (2001) reported a C/N range of 3.4–3.6 and 3.0–3.8, respectively. This wide range in C/N values for hair is not suitable to detect C contamination (as seen in the ^{14}C data mentioned above; Table S1). This is evident in the hair of subjects (A), (C), and (D) who are well within the C/N range described by O'Connell et al. (2001), but show evidence of ^{14}C contamination (Figure 2a,c,d). The ^{13}C nail-hair offset is lower by 0.3–0.4‰, and ^{15}N elevated by 0.4‰ (Figure S1; Figure S2a,c,d). Although we do not have an explanation for this offset, it has been suggested by O'Connell et al. (2001) that this could be due to amino acid compositional differences between nail and hair.

The hair of subject (B) is ^{13}C depleted relative to the nail by 0.8‰ (Figure S1b). The hair C/N values (~4.0–4.6) and the total %C (45.8–50.4) of subject (B) are elevated. In contrast, the observed %N (12.0–13.8) is lower compared to the other subjects (Table S1). Hair care products measured (one styling gel, one permanent coloring from a dark-brown dye kit, and two shampoos, including the infant/toddler mentioned earlier) were 42 to 78% C and displayed a $\delta^{13}C$ range of –31.5 to –29.0‰ (n = 13). These values reflect the use of petroleum-based distillates (which are ^{14}C-free), which generally display ^{13}C-depleted values (–50 to –23‰; Meier-Augenstein 1999). No useful information was obtained for nitrogen, as it appears that volatile N compounds were removed during the evaporation of hair care products before the measurement was performed.

The presence of fossil-fuel-derived C contamination in the hair of subject (B) is clearly confirmed by the ^{14}C results (Figure 2b); however, we cannot determine if fossil fuel compounds from cosmetic treatments may result in a loss of hair nitrogen as well. An alternative explanation for this apparent nitrogen loss could be the use of protein hydrolysates (derived from plants and animals)

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in commercial shampoos and conditioners (Jones and Chahal 1997; Villa et al. 2013 and reference therein). A significant percentage (>50%) of protein hydrolysates are able to penetrate into the hair structure (Jones and Chahal 1997). Therefore, it is difficult to determine the amount and type of contaminants (fossil fuel compounds and/or proteins hydrolysates) and chemical mechanism (gain, replacement, and losses of C and N) involved in their assimilation onto/into the hair matrix without further systematic analyses.

Subject (A) possibly demonstrates the effects of protein hydrolysates on human hair. The %C values for the hair of subject (A) that are farther from the scalp are elevated when compared to more recently formed hair. These portions of hair have had additional time to accumulate these protein hydrolysates into their structure (Jones and Chahal 1997). With an increase of C contamination, the C/N values (~3.5–3.7) are elevated towards the higher end of the acceptable C/N (3.8) ratio set by O'Connell et al. (2001). This contamination would go unnoticed if not for the longitudinal comparisons (%C, %N, C/N) done for subject (A) (Table S1).

Although diet reconstruction was not in the scope of this research, the general diet-related stable isotope trends can still be observed and will be discussed below. Isotopic results agree with the general food intake of the subjects and their expected trophic level position in the food chain (Table 1). Subject (A) is pescatarian, but her major protein consumption comes from a large mixture of C_3- and C_4-based products (wheat, soybean, dairy, and local vegetables). Subjects (B) and (C) share a household, and consequently their basic diet is somewhat similar, except for the overall quantities. They both consume meat (red and white), dairy products, rice, pasta, fruits, and vegetables. However, subject (B) limits the consumption of red meat (mostly beef), primarily consuming large amounts of dairy products, grains, chicken, and some seafood, while subject C does the opposite (more red meat, less dairy products, and <5% seafood). Subject (D) consumes predominantly beef and corn-based products.

Specialized Chemical Pretreatments

In order to determine if more aggressive chemical pretreatments can remove foreign carbon contamination present on and within the hair strands (especially for hair dye samples), we applied several dye removal procedures, including a commercial hair dye remover. It is uncertain whether dye removal products eliminate the dye from the hair or simply neutralize the pigments within the dye itself. After the application of the dye remover, detergent was applied to strip off the hair scales, exposing the cortex, and assist in the removal of any persistent cosmetic residues (Taylor et al. 1995). Hydrogen peroxide (3%) was also used due to its bleaching properties. The specialized treatments are outlined in Table 2, and results are depicted in Figure 3. However, the specialized pretreated hair ^{14}C results did not show any significant improvement compared to the ABA chemical pretreatment (i.e. the hair ^{14}C values are farther from the nail reference). We suspect that the specialized pretreatments applied left remnants of the most-aged exogenous C from the dye, which might explain the decrease in ^{14}C values. Further analyses are needed to confirm this hypothesis.

SEM images were obtained from various hair pretreatments. Figure 4a shows the hair surface before chemical pretreatments were applied. After the application of ABA and detergent, the hair cuticles appear intact (Figure 4b). While surface morphology between ABA-pretreated and untreated hair is indistinguishable, the hair scales after dye removal or hydrogen peroxide treatments are clearly damaged (Figure 4c,d). Nevertheless, it is apparent that the scales were not entirely removed (even when the detergent procedure was repeated 3 times). In addition, Figure 3 indicates that exogenous carbon from the specialized chemical pretreatments was deposited onto the cuticle layers, and this accounted for the larger ^{14}C offsets than the standard ABA pretreatment.

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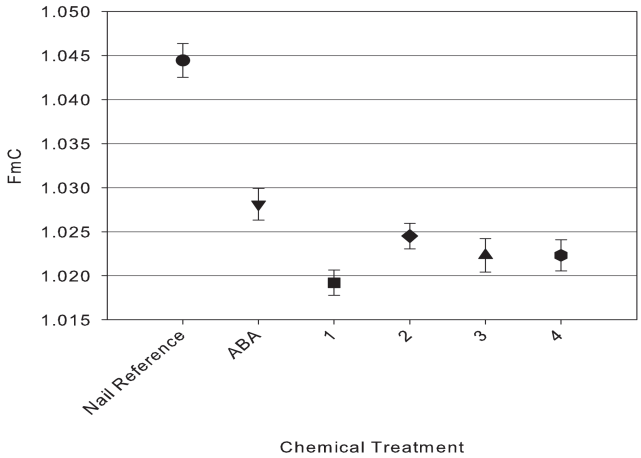

Figure 3 Average FmC hair values of subject B that have undergone specialized chemical pretreatments. The ^{14}C nail-hair offsets are (a) 16.3‰ for an ABA treatment; (b) 25.2‰ for treatment #1; (c) 19.9‰ for treatment #2; (d) 22.1‰ for treatment #3; and (e) 22.1‰ for treatment #4. Further information regarding specialized chemical pretreatments is described in Table 2.

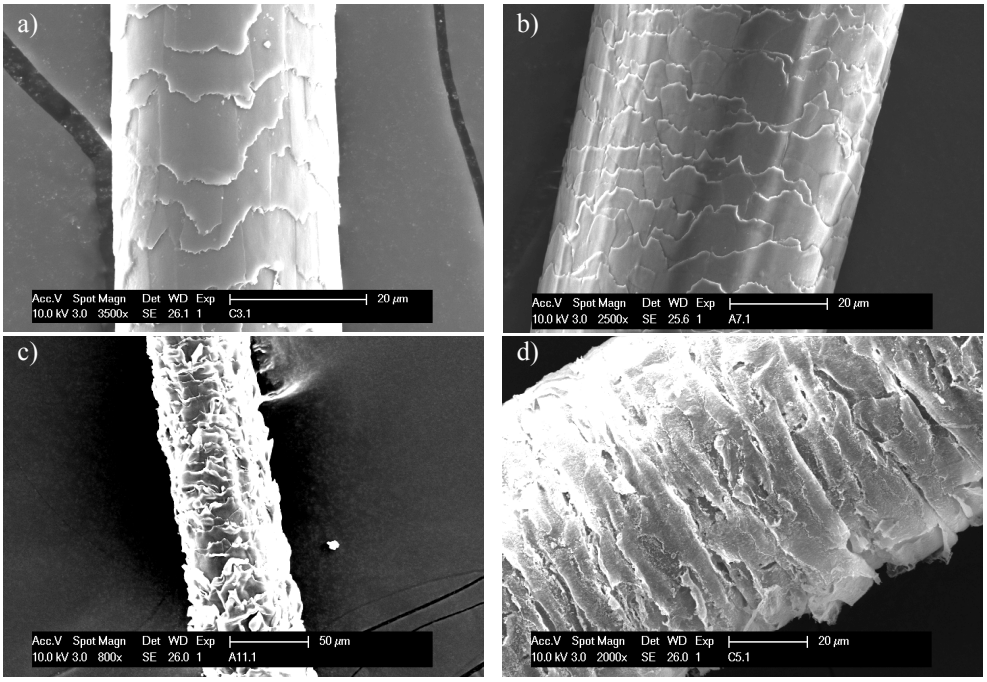

Figure 4 SEM images of hair strands after chemical pretreatments, clockwise from top left: (a) untreated hair strand (3500× magnification); (b) ABA-treated hair strand coupled with detergent washes (2500× magnification); (c) hair strand subjected to dye remover, detergent, and ABA (treatment #2; Table 2) shows significant damage but not complete removal of scales (800× magnification); and (d) hair strand subjected to hydrogen peroxide (3%) pretreatment (treatment #4; Table 2) also displays scale breakage due to disintegration of the sulfide bonds within keratin (2000× magnification).

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Human vs. Rat Prospective

To investigate whether anomalous C/N results from human nail-hair parings are due to isotopic fractionation along with the observed discrepancies in ^{14}C values, we tested nails and hair of rats for comparison (Table S1). The control group was comprised of adult rats, which were not subjected to cosmetic products and were raised on a commercial diet. Due to the short length of the rat hair, bulk isotopic analyses were performed and measured in duplicate. The control group samples underwent the same chemical cleaning procedures as the human subjects (e.g. ABA for hair and acetone/ethanol/MQ water for nails).

The ^{14}C results of the humans and rats are plotted in Figure 5a,b. Note that we plotted average ^{14}C values of human hair segments against single bulk human fingernail measurements, and mean bulk rat hair and nail values. Contrary to the ^{14}C results obtained from human nail-hair parings, the rats show no ^{14}C differences between keratin tissues (Table S1). Rat ^{14}C values depict a much tighter spread than the human subjects. All nail and hair ^{14}C results overlap within uncertainties ($\pm 2\sigma$) and range from 1.0359 to 1.0404 FmC. The average atomic C/N values are 3.4 ± 0.2 ($n = 3$) for nails and 3.6 ± 0.1 ($n = 3$) for hair. In addition, rat $\delta^{13}C$ values were within the analytical error of the mass spectrometer ($\pm 0.1‰$). However, the rat $\delta^{15}N$ values were more varied, and this might be attributed to individual food selection practices (Table S1).

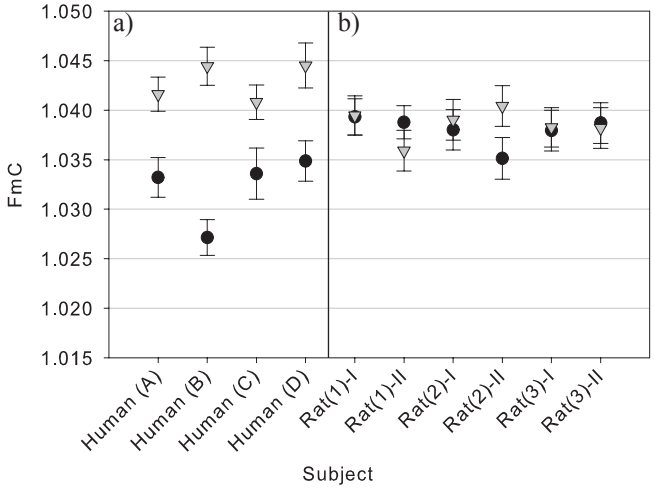

Figure 5 ^{14}C FmC values of keratinous tissues from humans and rats are shown in panels (a) and b). Roman numerals represent duplicate analyses of rat hair and nails from the same rat.

In order to evaluate whether all nail and hair FmC results closely followed the ^{14}C atmospheric values, we plotted our keratin tissue results from all human subjects and rats against an extrapolation of the Northern Hemisphere (NH; 40°N latitude and regions close to the Equator that follow the summer intertropical convergence zone) atmospheric ^{14}C curve (Figure 6). This curve includes measurements from air and tree rings, and it is believed to closely represent the present atmospheric ^{14}C values for the NH (Hua et al. 2013). A linear regression line was calculated with Sigmaplot, using the atmospheric ^{14}C data set from 1999 to 2010. For clarity, Figure 6 only shows atmospheric data between 2003 and 2010. An R^2 value was also determined (0.96) and a 95% confidence interval was added to the figure to account for any variability associated with seasonal differences.

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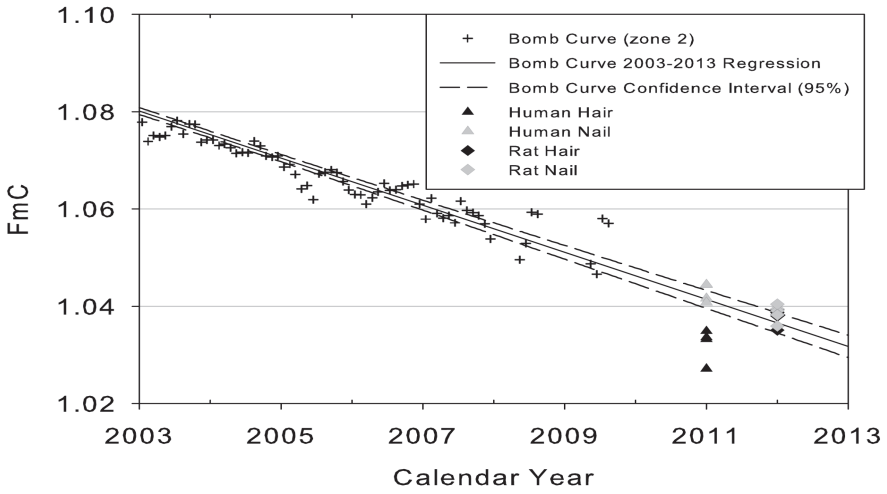

Figure 6 Average results for all humans and rats are plotted against the atmospheric ^{14}C bomb curve. All human hair measurements represent an average bulk value of the hair subsections, whereas the human nail is a single bulk value (Table S1). For the rat hair and nails, we averaged the ^{14}C results of bulk values obtained (as also shown in Table S1).

All subjects have nail ^{14}C results that are closer to the extrapolated regression curve for the timeframe when the samples were collected. In contrast, the human hair values are ^{14}C depleted, which can lead to erroneous year-of-death calendar ages after calibration (especially on the downslope of the bomb peak after 1964 to 2000). The human keratinous ^{14}C values were not corrected for the time-lag associated with the uptake of atmospheric ^{14}C by plants via photosynthesis, propagation through the food chain and/or storage, and the formation of new tissues (Hodgins 2009). Nevertheless, those facts should not affect our interpretation of the data. In contrast, the rat ^{14}C nail-hair pairing results are a perfect match to the timeframe of sampling (within uncertainties). This simple test was not intended to be exhaustive, but rather to show how our data agree overall with the present NH compilation data set for the region studied (Hua et al. 2013).

Our results, which make use of ^{14}C AMS measurements coupled with $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C/N values, support the finding that foreign C contamination (likely from fossil-fuel-derived products in hair treatment products) is biasing ^{14}C ages and ^{13}C stable isotope results. This fossil fuel contamination appears to be dominant, although some N contamination cannot be completely excluded, as some hair-care products used on a daily basis by humans also contain plant and animal proteins (Jones and Chahal 1997; Villa et al. 2013). So far, all hair pretreatment methods used within this research failed to achieve desirable results (O'Connell and Hedges 1999, ABA and specialized chemical pretreatments), as we demonstrated that contaminates are not just superficially associated with the hair strands (O'Connell and Hedges 1999; O'Connell et al. 2001). The assumption that the bulk stable isotope nail-hair offsets are simply due to the isotopic composition of individual amino acids (O'Connell et al. 2001) will remain open until efficient chemical pretreatments are developed to remove all foreign contaminates from hair.

CONCLUSION AND PROSPECTIVE

From our research, the anomalous offsets between nail and hair in modern human subjects are mostly due to fossil fuel carbon contamination onto/into hair strands. Since cosmetic products such as hair dyes, shampoos, and conditioners are designed to open hair scales and allow foreign substances

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to become trapped, this finding is not surprising. The nail-hair ^{14}C offsets as a result of C contamination due to hair care products in modern humans is validated by the lack of an observed difference in the nails and hair of modern rats, which do not make use of cosmetic products. Although the number of samples tested in the control group was limited, the ^{14}C AMS measurements and stable isotopic values are consistently reproducible.

The ^{14}C results from specialized chemical pretreatments applied to chemically treated hair (hair coloring) also showed ^{14}C-depleted values when compared to the fingernails. The SEM images showed that the specialized pretreatments did not remove the hair scales, and therefore did not help to eliminate foreign carbon from the hair strands. Thus, human hair strands are not suitable for ^{14}C determination of the year-of-death in forensic science cases until further chemical pretreatments are developed to effectively remove fossil fuel C contamination. In addition, C and N contamination from protein hydrolysates (from botanical and animal extracts) in cosmetic products may also play an important role in affecting stable isotope values, although the latter is still in need of further investigation. We conclude, if available, human fingernails should be the tissue of choice in the fields of forensics and dietary studies.

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REFERENCES

Arneborg J, Heinemeier J, Lynnerup N, Nielsen HL, Rud N, Sveinbjörnsdóttir ÁE. 1999. Change of diet of the Greenland Vikings determined from stable carbon isotope analysis and ^{14}C dating of their bones. *Radiocarbon* 41(2):157–68.

Auerswald K, Rossmann A, Schäufele R, Schwertl M, Monahan FJ, Schnyder H. 2011. Does natural weathering change the stable isotope composition (^{2}H, ^{13}C, ^{15}N, ^{18}O and ^{34}S) of cattle hair? *Rapid Communications in Mass Spectrometry* 25(24):3741–8.

Bergfeld W, Mulinari-Brenner F. 2001. Shedding: how to manage a common cause of hair loss. *Cleveland Clinic Journal of Medicine* 68(3):256–61.

Beverly RK, Beaumont W, Tauz D, Ormsby KM, von Reden KF, Santos GM, Southon JR. 2010. The Keck Carbon Cycle AMS Laboratory, University of California Irvine: status report. *Radiocarbon* 52(2):301–9.

Chen B-J, Lee P-L, Chen W-Y, Mai F-D, Ling Y-C. 2006. Hair dye distribution in human hair by ToF-SIMS. *Applied Surface Science* 252(19):6786–8.

Clausen T, Schwan-Jonczyk A, Lang G, Schuh W, Liebscher KD, Springob C, Franzke M, Balzer W, Imhoff S, Maresch G. 2006. Hair preparations. In: *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley VCH. doi: 10.1002/14356007.a12_571.pub2.

Contrino J, Hair G, Kreutzer DL, Rickles FR. 1996. In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. *Nature Medicine* 2(2):209–15.

DeNiro MJ. 1985. Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317(6040):806–9.

Evans T, Wickett RR. 2012. *Practical Modern Hair Science*. Carol Stream: Allured Business Media. 562 p.

Feigenbaum H. 2009. *The Use of Cationizing Reagents in the Preparation of Conditioning Polymers for Hair and Skin Care*. Saddle Brook: SKW QUAB Chemicals.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges RE. 2004. Nitrogen balance and $\delta^{15}N$: why you're not what you eat during pregnancy. *Rapid Communications in Mass Spectrometry* 18(23):2889–96.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges RE. 2005. Nitrogen balance and $\delta^{15}N$: why you're not what you eat during nutritional stress. *Rapid Communications in Mass Spectrometry* 19(18):2497–506.

Hao J, Li SK. 2008. Mechanistic study of electroosmotic transport across hydrated nail plates: effects of pH and ionic strength. *Journal of Pharmaceutical Sciences* 97(12):5186–97.

Hodgins GW. 2009. Measuring atomic bomb-derived ^{14}C levels in human remains to determine year of birth and/or year of death. NIJ Report, 2009. https://www.ncjrs.gov/pdffiles1/nij/grants/227839.pdf.

Hopps HC. 1977. The biologic bases for using hair and nail for analyses of trace elements. *Science of the Total Environment* 7(1):71–89.

Hua Q, Barbetti M, Rakowski AZ. 2013. Atmospheric radiocarbon for the period 1950–2010. *Radiocarbon* 55(4):2059–72.

Hunting AL. 1983. *Encyclopedia of Shampoo Ingredients*. Cranford, NJ: Micelle Press. 480 p.

Hunting AL. 1987. *Encyclopedia of Conditioning Rinse Ingredients*. Cranford: Micelle Press. 506 p.

Jones R, Chahal S. 1997. The use of radiolabelling techniques to measure substantivity to, and penetration into, hair of protein hydrolysates. *International Journal of Cosmetic Science* 19(5):215–26.

Kuzuhara A, Hori T. 2003. New method of dyeing keratin fibers using poly (ethylene imine) and its coloring mechanism. *Journal of Applied Polymer Science* 90(14):3806–10.

Langbein L, Rogers MA, Winter H, Praetzel S, Beckhaus U, Rackwitz H-R, Schweizer J. 1999. The catalog of human hair keratins. *Journal of Biological Chemistry* 274(28):19,874–84.

Lehn C, Mutzel E, Rossmann A. 2011. Multi-element stable isotope analysis of H, C, N and S in hair and nails of contemporary human remains. *International Journal of Legal Medicine* 125(5):695–706.

Lynnerup N, Kjeldsen H, Zweihoff R, Heegaard S, Jacobsen C, Heinemeier J. 2010. Ascertaining year of birth/age at death in forensic cases: a review of conventional methods and methods allowing for absolute chronology. *Forensic Science International* 201(1–3):74–8.

Mainkar A, Jolly C. 2001. Formulation of natural shampoos. *International Journal of Cosmetic Science* 23(1):59–62.

Meier-Augenstein W. 1999. Applied gas chromatography coupled to isotope ratio mass spectrometry. *Journal of Chromatography A* 842(1):351–71.

Meier-Augenstein W. 2011. *Stable Isotope Forensics: An Introduction to the Forensic Application of Stable Isotope Analysis*. New York: John Wiley & Sons. 296 p.

Nardoto GB, Silva S, Kendall C, Ehleringer JR, Chesson LA, Ferraz ES, Moreira MZ, Ometto JP, Martinelli LA. 2006. Geographical patterns of human diet derived from stable-isotope analysis of fingernails. *American Journal of Physical Anthropology* 131(1):137–46.

O'Connell TC, Hedges RE. 1999. Investigations into the effect of diet on modern human hair isotopic values. *American Journal of Physical Anthropology* 108(4):409–25.

O'Connell TC, Hedges REM, Healey MA, Simpson AHRW. 2001. Isotopic comparison of hair, nail and bone: modern analyses. *Journal of Archaeological Science* 28(11):1247–55.

Odland G, Goldsmith I. 1991. *Physiology, Biochemistry and Molecular Biology of the Skin*. Oxford: Oxford University Press. Volume 1. p 3.

Oshimura E, Abe H, Oota R. 2007. Hair and amino acids: the interactions and the effects. *Journal of Cosmetic Science* 58(4):347–57.

Reimer PJ, Brown TA, Reimer RW. 2004. Discussion: reporting and calibration of post-bomb ^{14}C data. *Radiocarbon* 46(3):1299–304.

Santos GM, Ormsby K. 2013. Behavioral variability in ABA chemical pretreatment close to the ^{14}C age limit. *Radiocarbon* 55(2):534–44.

Santos GM, Moore RB, Southon JR, Griffin S, Hinger E, Zhang D. 2007. AMS ^{14}C sample preparation at the KCCAMS/UCI Facility: status report and performance of small samples. *Radiocarbon* 49(2):255–69.

Santos GM, Ormsby K, Fuller B. 2011. Can we blame discrepancies of year-of-birth ^{14}C determination on mom? Preliminary isotope results of fingernails of breastfed mother-infant pairs. In: *Programme and Abstracts Handbook of the AMS-12 (The Twelfth International Conference on Accelerator Mass Spectrometry)*. Wellington, New Zealand. p 176.

Schwarcz HP, Schoeninger MJ. 1991. Stable isotope analyses in human nutritional ecology. *American Journal of Physical Anthropology* 34(S13):283–321.

Stenström K, Unkel I, Nilsson CM, Rääf C, Mattsson S. 2010. The use of hair as an indicator of occupational ^{14}C contamination. *Radiation and Environmental Biophysics* 49(1):97–107.

Taylor R, Hare P, Prior CA, Kirner DL, Lijun W, Burky RR. 1995. Radiocarbon dating of biochemically characterized hair. *Radiocarbon* 37(2):319–30.

Tsanaclis L, Wicks J. 2008. Differentiation between drug use and environmental contamination when testing for drugs in hair. *Forensic Science International* 176(1):19–22.

van Klinken GJ, Hedges REM. 1995. Experiments on collagen-humic interactions: speed of humic uptake, and effects of diverse chemical treatments. *Journal of Archaeological Science* 22(2):263–70.

Villa AL, Aragão MR, dos Santos EP, Mazotto AM, Zingali RB, de Souza EP, Vermelho AB. 2013. Feather keratin hydrolysates obtained from microbial keratinases: effect on hair fiber. *BMC Biotechnology* 13(1):15, doi:10.1186/1472-6750-13-15.

Von Braun J, Diaz-Bonilla E. 2008. *Globalization of Food and Agriculture and the Poor (No. 52)*. International Food Policy Research Institute (IFPRI). Oxford: Oxford University Press.

Wennig R. 2000. Potential problems with the interpretation of hair analysis results. *Forensic Science International* 107(1):5–12.

Wild EM, Arlamovsky KA, Golser R, Kutschera W, Priller A, Puchegger S, Rom W, Steier P, Vycudilik W. 2000. ^{14}C dating with the bomb peak: an application to forensic medicine. *Nuclear Instruments and Methods in Physics Research B* 172(1):944–50.

Wilson A, Dodson H, Janaway R, Pollard A, Tobin D. 2010. Evaluating histological methods for assessing hair fibre degradation. *Archaeometry* 52(3):467–81.

Zaias N. 1990. *The Nail in Health and Disease*. Norwalk: Appleton & Lange. 181 p.