Fatty acid composition and preservation of the Tyrolean Iceman and other mummies

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Abstract In anthropology, objective parameters to adequately describe storage conditions and the preservation of mummies have yet to be identified. Considering that fatty acids degrade to stable products, we analysed their profile in human mummies and in control samples by gas chromatography coupled to mass spectrometry (GC/MS). Originating from different epochs and civilizations, samples of the Tyrolean Iceman, other glacier corpses, a freeze dried mummy, corpses from a permafrost region, a corpse mummified immersed in water, and a desert mummy were evaluated. Chemometric analysis based on the concentrations of 16 fatty acids revealed the degree of similarity between anthropologic and fresh corpse samples, which was mainly influenced by the content of palmitic acid, oleic acid, and 10-hydroxystearic acid. The presence of 10-hydroxystearic acid was associated with immersion in water, whereas dry mummification was accompanied by high contents of oleic acid. Samples of the Tyrolean Iceman clustered between fresh tissue and those of other glacier corpses indicating the good preservation of this mummy. Thus, environmental post-mortem conditions were associated with characteristic fatty acid patterns suggesting that chemometric analysis of fatty acid contents may add to our knowledge about post-mortem storage conditions and the preservation of human corpses.—Makristathis, A., J. Schwarzmeier, R. M. Mader, K. Varmuza, I. Simonitsch, J. Chavez Chavez, W. Platzer, H. Unterdorfer, R. Scheithauer, A. Derevianko, and H. Seidler. **Fatty acid composition and preservation of the Tyrolean Iceman and other mummies. *J. Lipid Res.* 2002.** 43: **2056–2061.**

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In September 1991, an approximately 5,000-year-old frozen male mummy was found in the Similaun glacier of the Tyrolean Alps (1). Interestingly, the subcutaneous tissue and the adipose tissue of the so-called Tyrolean Iceman appeared macroscopically better preserved than tissue of other corpses buried in glaciers for much shorter periods of time. Consecutive studies, however, showed an almost complete degradation of macromolecules (2). This indicated the need for more appropriate methods to adequately describe post-mortem alterations of anthropologic finds. Since fatty acids are small molecules with defined degradation products, the study of these components is more likely to reflect the influence of environmental conditions on post-mortem alterations (3, 4).

Post-mortem, body fat is converted into adipocere under humid and microaerobic conditions. Adipocere is a lipid mixture of wax-like consistency and greyish-white color consisting mainly of free saturated fatty acids with even numbers of carbon atoms and eventually hydroxy-fatty acids. The formation of the latter has been attributed to biotic as well as to abiotic processes (5, 6). On the other hand, air circulation and/or elevated temperatures lead to mummification of human tissue by means of desiccation. Under these conditions, the epidermis becomes tanned thus protecting the tissue underneath. A rapid desiccation process is often associated with macroscopically well-preserved tissue.

To learn more about the millennial preservation of the Tyrolean Iceman, we analysed the fatty acid composition of Iceman's tissue specimens by gas chromatography cou-

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pled to mass spectrometry (GC/MS). Using chemometric methods, these data were compared with those of other well-preserved mummies from different epochs and civilisations exposed to defined climatic conditions.

MATERIALS AND METHODS

Human samples

Specimens obtained from the Tyrolean Iceman, two other corpses found in glaciers nearby, a body permanently immersed in an Austrian mountain lake over 50 years, two Scythian corpses buried in the permafrost of Siberia, a freeze dried Inca mummy from the Peruvian Andes, and, finally, a mummy buried in the Peruvian desert were evaluated (**Table 1**). As reference, the fatty acid profile of fresh tissue samples from three recently deceased control subjects were evaluated (17 specimens including skin, muscle, bone marrow, lung, and liver).

Analytical procedure

In order to saponify the lipid material, tissue samples were homogenised and treated for 30 min at 100°C with 1 ml of a mixture of 7.5 N sodium hydroxide (Merck, Darmstadt, Germany) and methanol (1:1, v/v; Merck). The sodium salts of the free fatty acids were converted to their methyl esters by adding 2 ml of a mixture of methanol and 6 N hydrochloric acid (4.6:5.4, v/v; Merck) and heating for 10 min at a temperature of 80°C. Fatty acid methyl esters were then transferred from the acidic aqueous phase to an organic phase by liquid-liquid extraction using 1.25 ml of a mixture of n-hexane and t-butylethylether (1:1, v/v; Merck). Finally, cleanup of the organic extract was performed by liquid-liquid extraction using 3 ml of a 0.3 N sodium hydroxide solution. All reagents were of analytical grade. This extraction protocol—first described by Sasser (7)—allows for the analysis of the whole fatty acid content of tissue specimens including bound and unbound fatty acids, and also those originating from sources other than lipids (e.g., lipoproteins). Considering the small amount of the available ancient samples, this approach may maximize the recovery of fatty acids.

The extracts were subjected to qualitative and quantitative analysis twice by gas-liquid chromatography (Hewlett Packard 5890, Agilent Technologies, Waldbronn, Germany) using a capillary column (Hewlett Packard Ultra 2; 25 m × 0.2 mm × 0.33 μm film thickness with 5% phenyl methyl silicone as stationary phase) coupled to a mass spectrometer (Finnigan 8200, Bremen, Germany). Species resolved by gas chromatography were identified by mass spectrometry using the database system MassLib (Max-Planck Institut für Kohlenforschung, Mülheim an der Ruhr, Germany).

Data analysis

In order to visualize the relationships within the fatty acid data set, principal component analysis (PCA) was applied (8). This standard technique of exploratory multivariate data analysis was chosen due to the composition of the available data set that, because of the small number of samples and the lack of replicates, can not be evaluated by elementary statistical tests. More importantly, this advantageous multivariate approach uses a set of variables instead of a single variable for the description of similarities between samples.

The concentrations of 16 selected fatty acids (threshold: 1% of total fatty acids) were used as features to characterize a sample. In order to eliminate the influence of absolute concentration values, the features were autoscaled (to a mean of zero and a variance of 1) before PCA. The resulting first and second principal component scores (each a linear combination of the concentrations of the 16 fatty acids) were used as coordinates for a scatter plot with a point for each sample. Visual inspection of this score plot shows clustering according to the similarity of samples. In a loading plot, the principal component loadings were used as coordinates for points that correspond to the features (fatty acids). A fatty acid, which for instance is located in the upper right hand corner, is characteristic for samples located in the same region of the score plot. Fatty acids with a large distance from the origin of the coordinate system possess highest influence on the data set. The results obtained from PCA were confirmed by cluster analysis using dendrograms, and by k-nearest neighbour classifications. These methods extract relevant information from a data matrix and are useful in the interpretation of results. However, the small data set did not allow for the estimation of statistical validity. The software used was SCAN (Minitab Inc., State College, PA).

RESULTS

As shown in **Fig. 1A**, unsaturated fatty acids and palmitic acid dominated the fatty acid profile of fresh tissue. The concentration of unsaturated fatty acids, predominantly oleic acid (18:1), was higher in specimens of the Tyrolean Iceman (Fig. 1B; **Table 2**, line A) than in samples from the other corpses found in glaciers nearby (Fig. 1C; Table 2, lines B and C). With concentrations up to 49%, the amount of hydroxy stearic acid (18:0 10OH) was similar in both the Tyrolean Iceman and the other two glacier corpses (Fig. 1B, C; Table 2).

The influence of continuous immersion into cold water on post-mortem degradation was studied by analysing the fatty wax of a body recovered 50 years after death from a mountain lake in a depth of 50 meters. The fatty acid composition of samples obtained from this corpse (Table 2, line D) differed from the recently buried glacier corpses mainly in the higher concentrations of myristic acid (range: 15–21% of total fatty acids vs. 2.8–8.5% in glacier corpses).

The fatty acid profiles obtained from two corpses buried in the Altai mountains differed between each other (Pazyryk culture in Siberia). One of them, a Scythian warrior, was excavated as a frozen mummy in a wooden coffin filled with ice. The fatty acid composition of this specimen was similar to that observed in specimens of glacier corpses with 10-hydroxystearic acid as the dominant component (45% of total fatty acids; Table 2, line E). After burial, the other corpse decayed in the permanent frost without being enclosed in ice. A specimen from the pelvic region was one of the few tissue samples conserved on this skeleton. In contrast to the specimen obtained from the Scythian warrior, the fatty acid profile of this sample consisted predominantly of saturated fatty acids (Table 2, line F).

Two specimens originating from a freeze-dried mummy found in a cavern of the Peruvian Andes (Mount Ampato) were also evaluated. Perhaps sacrificed by the Incas for religious motives ~500 years ago, this well preserved mummy of a child was desiccated by winds at an altitude of 6,000 m. A remarkably high concentration of unsaturated fatty acids was observed (23.5 and 29% of total fatty

TABLE 1. Origin, storage conditions, burial time, and specimens of the evaluated human mummies and control subjects

ID	Origin and Storage Conditions	Burial Time	Probable Age at Death	Specimen (Weight)[a]
		years		*mg*
A	"Tyrolean Iceman", glacier Similaun, South Tyrol, Italy; 3,200 m altitude; male corpse probably exposed to weather immediately after death, then stored in ice, found in melting ice in 1991.	~5,200	35–40	A1: trabecular bone (11), A2: nasal cavity (8.8); A3: paranasal sinus (8.2); A4: skin left hip (13).
B	Glacier Madatschferner, Austria; 2,800 m altitude; female corpse initially buried in the glacier, after melting of ice likely to be immersed in water for several months, found uncovered on ground free of snow in 1952.	29	28	B1: muscle right calf (15); B2: skin right thigh (17); B3: left lung surfacial adipocere (9.4); B4: liver surfacial tissue (10); B5: liver internal tissue (12).
C	Glacier Sulztalferner, Austria; 2,700 m altitude; male corpse partially buried in the glacier, likely to be immersed in melting ice for several months, found uncovered on ice in 1991.	57	62	C1: muscle upper left arm (17); C2: liver internal tissue (13); C3: skin abdomen (15); C4: skin with fat and muscle radial section of upper arm (19).
D	Mountain lake Achensee, Austria; female corpse found in 50 m depth in 1989.	50	30	D1: left lung (17); D2: cardiac muscle (12); D3: muscle left thigh (14).
E	Altai Mountains, Siberia, Russia; 2,500 m altitude; male corpse buried in a permafrost zone, excavated completely enclosed in ice in 1995.	~2,200	Adult; detail information missing	E: skin abdomen (13).
F	Altai Mountains, Siberia, Russia; 2,500 m altitude; female skeleton with residual tissue found buried in a permafrost zone in 1993.	~2,500	Adult; detail information missing	F: tissue from the pelvic region (9.7).
G	Mount Ampato, Andes, Peru; 6,000 m altitude; female corpse dry frozen by mountain winds in a zone of eternal ice, found in 1995.	~500	8–10	G1: skin left temple (11); G2: hair left temple (9.1).
H	Ilo, Peru; male corpse mummified in a desert without rainfalls in the last millennium, found in 1988.	~1,000	Adult; detail information missing	H: muscle left lower leg (16).
I–K	Three fresh corpses as reference (two females and one male).		47, 72, and 90, respectively	I1: muscle abdomen (17); I2: muscle lower leg (23); I3: liver (15); I4: lung (18); I5: bone marrow (13); I6: skin with fat thigh (21); I7: skin with fat abdomen (19); J1: liver (13); J2: lung (23); J3: muscle abdomen (27); J4: skin with fat thigh (21); J5: skin with fat abdomen (19); K1: liver (22); K2: lung (15); K3: muscle abdomen (25); K4: skin with fat thigh (28); K5: skin with fat abdomen (21).

[a] Because of the unique nature of ancient specimens, it was not possible to obtain larger or multiple samples.

acids), whereas hydroxy stearic acid was completely absent (Table 2, line G). Only in these samples was a ramified fatty acid with 15 carbon atoms detected (11% and 15% of the total fatty acids).

Mummification under the complete absence of humidity has been investigated by analysing a tissue sample from a burial place near Ilo in the Peruvian desert (Chiribaya culture, 1,000 years old). In this place, precipitation has been unknown for thousands of years. This specimen showed almost all characteristics of fresh tissue, differing only in the reduced concentration of linoleic acid (1.2% vs. 8.6–18% of total fatty acids) accompanied by an increased concentration of palmitoleic acid (19% vs. 2.7–10% of total fatty acids; Table 2, line H).

Chemometric data analysis by principal component analysis was performed to unravel similarities among samples based on their fatty acids composition. Such a multivariate approach may sufficiently discriminate samples, even if single variables do not show significant differences. The score plot (**Fig. 2A**) obtained from the first and second principal component shows distinct regions, which can be attributed to different sample groups. The most homogenous group consisted of the fresh tissue samples from three different corpses clustering totally different specimens such as skin, muscle, bone marrow, liver, and lung (I1–I7, J1–J5, K1–K5). This was not unexpected, because the univariate statistical analysis of the lead compounds palmitic acid, oleic acid, and linoleic acid showed a coefficient of variation of only 15% to 18% when considering all control samples (n = 17). Samples obtained from the desert corpse (H) and the Inca mummy (G1 and G2) were adjacent to the group of fresh specimens. Opposite to this cluster, samples of the glacier corpses B and C formed a second group. With exception of the skin speci-

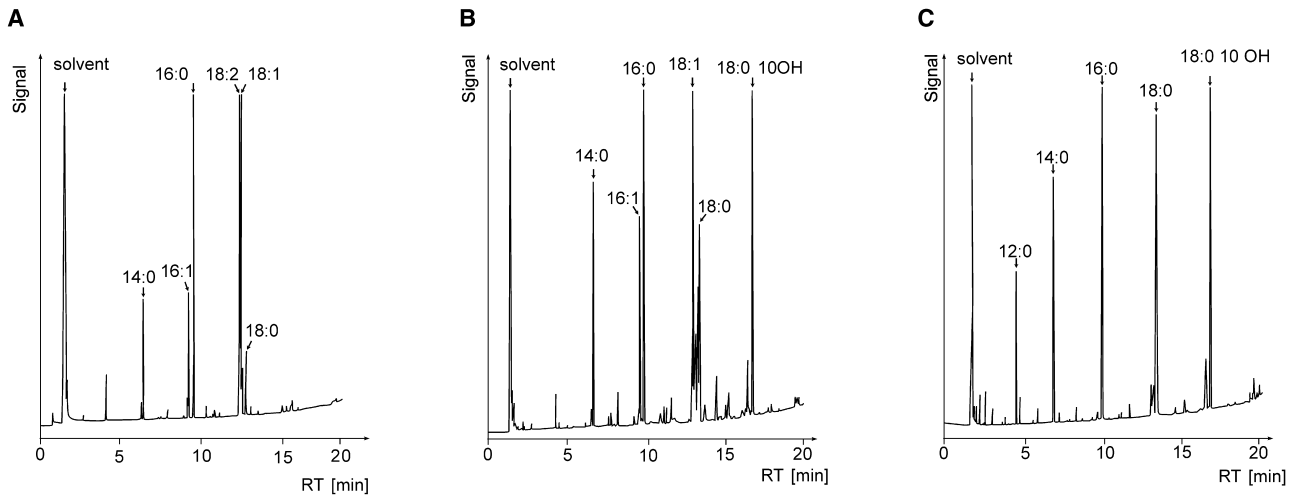

Fig. 1. Fatty acid profile of fresh and mummified tissue as analysed by gas chromatography demonstrating the greater affinity of the Tyrolean Iceman's fatty acid pattern to that of fresh tissue than that of another glacier corpse: A, skin with attached fat from a fresh human corpse (specimen I7, Table 1); B, trabecular bone of the Tyrolean Iceman (burial time: approximately 5,000 years; specimen A1, Table 1); C, liver recovered from a corpse buried in the glacier Madatschferner (burial time: 29 years; specimen B5, Table 1). Fatty acids are denoted as follows: 12:0, lauric acid; 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:0 10OH, 10-hydroxy stearic acid.

men (A4), the samples of the Tyrolean Iceman were distributed between these two groups (A1–A3). As demonstrated by the loading plot (Fig. 2B), the clustering of samples is mainly influenced by three components, namely oleic acid, 10-hydroxystearic acid, and palmitic acid. Considering these components in the Tyrolean Iceman, the skin (as the most exposed region of the corpse) differed from the other three samples (oleic acid, 8.7% vs. 12–22%; 10-hydroxystearic acid, 48% vs. 15–27%; palmitic acid, 18% vs. 20–36%).

DISCUSSION

The number of available ancient, well-preserved, and defined specimens may be very limited. This may include the type of recovered tissue as well as the amount of sample. As a prerequisite for the evaluation performed in the present study, one needs to establish the comparability of different tissue types. As shown in Fig. 2A, the most homogeneous group consisted of 17 specimens from three recently deceased subjects indicating that the type of tissue exerts only a minor influence with respect to their distribution on the score plot (samples I1-I7, J1-J5, K1-K5). Thus, the position of a sample on the score plot is mainly due to the environmental conditions it has been exposed to rather than to the sampling site of the corpse.

The samples from the two glacier corpses as well as those from the corpse recovered from a lake formed a second group (Fig. 2A; samples B1-B5, C1-C4, D1-D3). The specimens of the Tyrolean Iceman were distributed between these two groups (Fig. 2A, samples A1–A4). This finding was surprising and indicated the good preservation of this very old mummy in comparison to the corpses, which were buried in glaciers for much shorter time periods (29 and 57 years, respectively). The inhomogeneous distribution of the samples from the Tyrolean Iceman is likely due to the varying environmental conditions to which the different body regions were exposed during the millennial conservation process. Due to higher concentrations of 10-hydroxystearic acid and lower concentrations of oleic acid, the skin as the most exposed part of the Ty-

TABLE 2. Concentrations of the main components of the fatty acids in the evaluated human mummies and control subjects

	Human Mummies and Control Subjects										
Fatty Acids	A	B	C	D	E	F	G	H	I	J	K
Myristic acid (14:0)	2.8–7.0	3.2–8.5	2.8–6.2	15–21	6.0	5.5	3.3–4.2	4.1	<1–5.0	3.1–6.4	1.8–4.7
Palmitoleic acid (16:1)	0–5.0	n.d.	0–2.7	n.d.	1.1	n.d.	4.0–4.5	19	2.7–10	3.5–7.6	2.9–10
Palmitic acid (16:0)	18–36	21–37	36–50	35–46	25	55	30–33	22	17–30	22–32	25–30
Linoleic acid (18:2)	0–2.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.2	8.6–18	12–15	13–15
Oleic acid (18:1)	8.7–22	1.4–4.2	2.9–8	2.9–4.3	10	7.1	19–25	44	27–45	30–46	27–42
10-Hydroxypalmitic acid (16:0 10OH)	0–3.5	3.5–8.7	2–3.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Stearic acid (18:0)	5.0–17	3–19	3.4–7.4	4.4–9.9	6.3	26	8.7–14	1.8	2.5–11	2.5–7.1	2.2–10
Arachidonic acid (20:4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<1–9.4	<1–7.7	<1–12
10-Hydroxystearic acid (18:0 10OH)	15–48	21–49	7.5–43	9.8–34	45	n.d.	0–1.2	1.2	n.d.	n.d.	n.d.

Data represent the concentration range of the respective fatty acid. Results are calculated as percentage of the total fatty acid concentration (n.d., not detected). Letters A–K identify the human mummies and control subjects evaluated as indicated in Table 1.

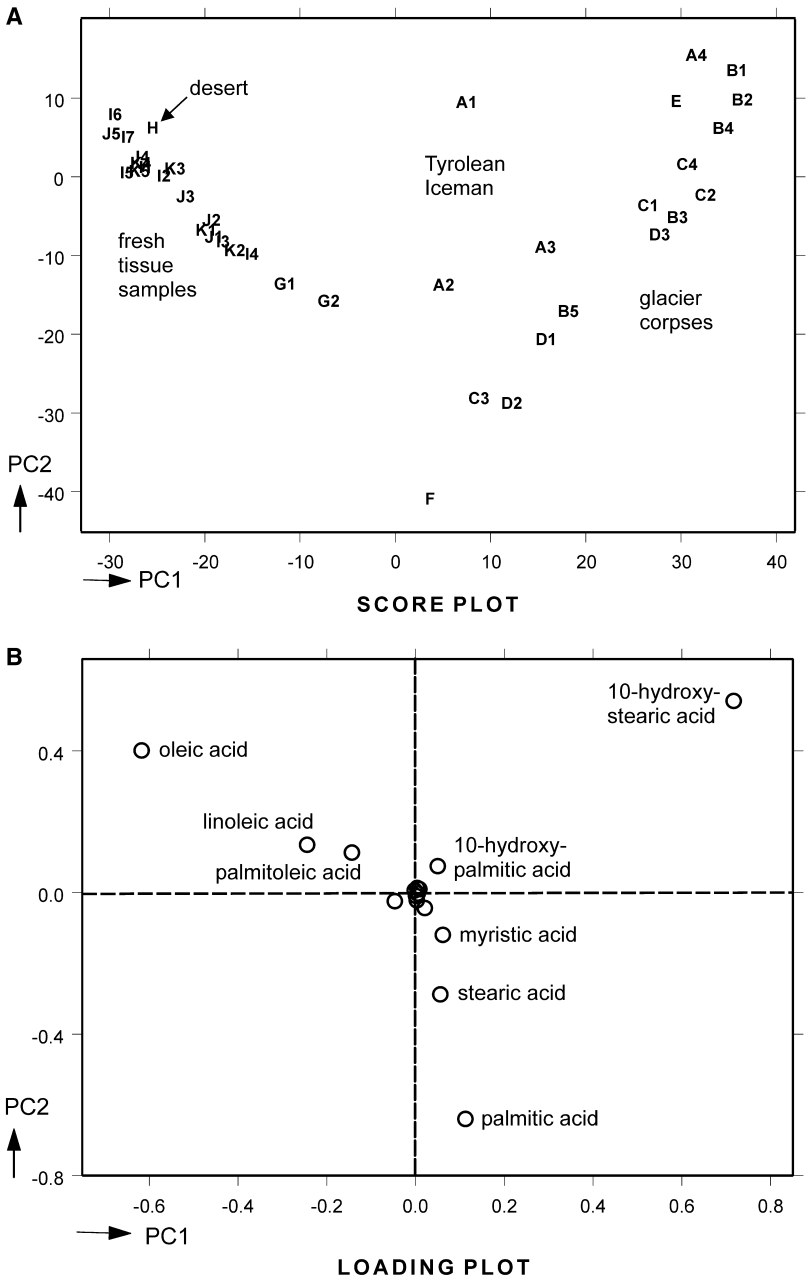

Fig. 2. Principal component analysis (PCA) of the autoscaled fatty acid data. PC1 is the first principal component (73.3% of total variance) and PC2 is the second principal component (18.3% of total variance); letters and subscripts indicate the sample identification as defined in Table 1; scaling of axes is linear in arbitrary units. A, score plot showing clustering of the 38 samples; B, loading plot showing the fatty acids that are characteristic for the clusters in the score plot.

rolean Iceman could hardly be distinguished from samples of the other glacier corpses (Fig. 2A, sample A4).

All samples from both the glacier corpses and the corpse recovered from the lake were characterized by the presence of 10-hydroxystearic acid, which was likely formed from the addition of a hydroxyl group to the double bond in oleic acid. This was conspicuous when considering the samples originating from the frozen Scythian corpses, which were found buried in the same area. The specimen of the Scythian warrior enclosed in ice was dominated by the presence of 10-hydroxystearic acid and was scattered within the glacier group on the score plot (Fig. 2A, sample E), whereas the sample of the Scythian skeleton was characterised by saturated fatty acids completely lacking 10-hydroxystearic acid (Fig. 2A, sample F). In contrast to the Scythian skeleton, the corpse of the Scythian warrior has been immersed in water (e.g., for several weeks during a hot summer), which then froze into ice. These findings suggest the formation of 10-hydroxystearic acid to be associated with watery storage conditions. This conclusion is supported by the presence of considerable amounts of this fatty acid in the body permanently im-

mersed in the mountain lake as well as in the glacier corpses, which were all temporarily exposed to water. Due to the very low storage temperatures of these corpses, the microbial contamination discussed in previous reports (6) is unlikely to play a major role in the formation of 10-hydroxystearic acid. The mechanism of the post-mortem fatty acid hydroxylation, however, remains to be elucidated.

In mammalian systems, detection of fatty acids with an odd number of carbon atoms is unusual. Thus, the relatively high concentrations of a ramified fatty acid with 15 carbon atoms in samples of the freeze-dried mummy from the Peruvian Andes was an unexpected finding. However, the authors cannot provide a reasonable explanation regarding the putative origin of this fatty acid. As expected from the absence of 10-hydroxystearic acid as well as the high concentrations of unsaturated fatty acids, the score plot grouped the samples of this mummy in immediate proximity to the fresh specimens (Fig. 2A, samples G1-G2). The best preserved corpse, however, was the one excavated in the Peruvian desert. The sample obtained from this mummy was congruent with fresh tissue on the score plot (**Fig. 2A**, sample H). Notwithstanding, even in this well-preserved specimen arachidonic acid was not detected which may be due to the unstable nature of this fatty acid.

We conclude that multivariate evaluation of fatty acid profiles of anthropologic samples from different epochs and civilizations characterized the respective preservation and allowed conclusions about the individual post-mortem storage conditions. The Tyrolean Iceman was found to be better preserved than corpses buried in neighboring glaciers for much shorter time periods. This can be explained by rapid initial desiccation (mummification) of the Tyrolean Iceman by mountain winds after his death, as suggested by macroscopic investigations (1). The corpse was probably then enclosed in ice, including periods of residence in water as indicated by the presence of 10-hydroxystearic acid, which is a putative marker for storage of anthropologic specimens in watery environment.

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