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TECHNICAL NOTE

PHYSICAL ANTHROPOLOGY

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Free Fatty Acids Composition in Adipocere of the Kwäday Dän Ts'ìnchí Ancient Remains Found in a Glacier*

ABSTRACT: Adipocere is a postmortem decomposition product consisting of mostly a mixture of free fatty acids (FFAs) that are formed because of the hydrolysis of triglycerides in adipose tissues. This article describes a simple and robust method for the extraction, identification, and quantification of FFA commonly found in adipocere using gas chromatography–mass spectrometry (GC/MS). This method was applied to analyze tissues from Kwäday Dän Ts'ìnchí, ancient remains discovered in a retreating glacier in the Tatshenshini-Alsek Park, British Columbia, Canada in August 1999. The lyophilized tissues were grinded and extracted with hexane. The trimethylsilyl fatty acid derivatives were analyzed by GC/MS, and the relative abundances of myristic acid, palmitic acid, oleic acid, and stearic acid were determined. Milligram per gram levels of saturated fatty acids were found in the tissues of the ancient remains, while the levels of unsaturated fatty acids, such as palmitoleic acid, were found to be negligible. The results provided further evidence of the existence of adipocere found during forensic examination of the Kwäday Dän Ts'ìnchí ancient remains.

KEYWORDS: forensic science, free fatty acids, adipocere, extraction method, gas chromatography–mass spectrometry, ancient human remains

On August 14, 1999, the frozen remains of a young man were discovered by hunters in a glacier in the Tatshenshini-Alsek Park of northwestern British Columbia (BC), Canada. The preserved body was the first ancient corpse discovered in a melting glacier in North America. The local Champagne and Aishihik First Nations people named the remains "Kwäday Dän Ts'ìnchí", which means Long-Ago Person Found. Several items were discovered with the ancient body, including a robe-style fur garment, a plant fiber hat, and some wooden artifacts. Initial radiocarbon dating of the plant fibers on his hat and the animal skin clothing indicated an age of about 500 years (1). However, more recent radiocarbon data obtained directly from the analysis of his body suggested that Kwäday Dän Ts'ìnchí more likely died between 1670 and 1850 AD (2), still predating Europeans entering this region (circa 1890). The local people allowed the collection of small amounts of samples enabling limited scientific research to continue after the cremation of the Kwäday Dän Ts'ìnchí, and these collected samples provided a unique opportunity to assess the tissues in frozen remains of this age for study (3–6).

The name adipocere is derived from Latin, meaning fatty wax, which is also known as grave wax or mortuary wax. This grayish-white water-insoluble material is the late-stage postmortem hydrolysis product of triglycerides in adipose tissues. A number of studies on the characterization of the chemical composition and properties of adipocere have been reported (7–13). The characterization of the preservation process provides important information about the surrounding environment in a forensic context. The conditions under which this process can occur vary, but humid and anaerobic conditions must be present. Corpses released by glaciers and bodies found in aquatic environments often have adipocere present. However, the formation of this substance is not well understood because of insufficient knowledge about the specific factors leading to adipocere. As such, it is of considerable interest to have a reliable analytical method that enables the identification and quantification of adipocere to evaluate the state of decomposition of a body and to assess the influence from the surrounding environment.

Beattie et al. (1) reported adipocere formation on the back, the vertebral spine, and the intrinsic muscles of Kwäday Dän Ts'ìnchí. The formation of adipocere usually takes about 3–6 months, but a complete transformation of the whole body could take many years (7). The formation of adipocere is initiated by intrinsic lipases, which convert the triacylglycerides (TAG) into their corresponding saturated and unsaturated fatty acids. The unsaturated fatty acids may then be hydrogenated, facilitated by degradative anaerobic bacteria, to form their corresponding saturated fatty acids in humid and micro-aerobic conditions (14).

The major constituents of adipocere have been identified as saturated free fatty acids (FFAs) with even numbers of carbon atoms, such as myristic acid, palmitic acid, and stearic acid. Among these, palmitic acid is usually the most abundant acid, followed by stearic acid then myristic acid (11,12). Unsaturated fatty acids, including palmitoleic, oleic, and linoleic acid, may be present in smaller amounts (15,16). In some cases, minor components such as TAG, 10-hydroxystearic acid, 12-hydroxystearic acid, phytanoic acid, and the calcium and magnesium salts of fatty acids are also found (8,15–21).

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TABLE 1—*Optimum detection conditions for GC/MS analysis of FFA.*

Parameter	Condition
GC parameters	
Column type	DB-5MS (J&W Scientific, Santa Clara, CA) Fused-silica capillary column (30 m × 0.25 mm × 0.25 μm 5% phenyl, 95% dimethylpolysiloxane)
Inlet pressure	100 kPa
Carrier gas	Helium
Injection volume	1 μL
Injection type	Pulsed splitless mode
Temperature	250°C
Initial oven temperature	100°C
Rate of change	15°C/min to 210°C and 5°C/min to 290°C held 3 min
MS parameters	
Acquisition mode	Scan
Scan parameters	50–650 *m/z*
Solvent delay	3 min
Quadrupole temperature	150°C
Source temperature	230°C

GC, gas chromatography; MS, mass spectrometry; FFA, free fatty acids.

There have been some methods reported that analyze the FFA components of adipocere. Stuart et al. and Bereuter et al. (15,17) have reported a rapid screening method to identify adipocere formation using infrared spectroscopy. A study of adipocere using high-performance liquid chromatography (HPLC) was demonstrated by Yan et al. (18). Gas chromatography (GC) and GC combined with mass spectrometry (GC/MS) were also used to the chemical characterization of adipocere (10,12). This work reports a rapid and simple method of extraction in combination with GC/MS for quantification of FFAs from adipocere tissues.

Materials and Methods

Chemicals and Materials

The protocol and consent form for the study of Kwäday Dän Ts'ìnchí was approved by the Clinical Research Board Ethics Committee at the University of British Columbia. Trapezius (0.5 g) and bicep (0.5 g) muscles from the Kwäday Dän Ts'ìnchí (kept at −80°C) and from an unembalmed male cadaver donated for education and research to the Faculty of Medicine at the University of British Columbia were excised with a sterile scalpel. The cadaver tissues, which also were stored at −80°C, were used as controls.

The lipid standards (GC grade), including triolein, myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, linoleic acid, oleic acid, and stearic acid, were purchased from Sigma–Aldrich (Oakville, ON, Canada), as was the silylating agent, *N*,*O*-bis(trimethylsilyl) trifluoroacetamide containing 1% v/vtrimethylchlorosilane (BSTFA + 1% TMCS). Individual stock solutions (10 mg/mL) of lipid standards were prepared in hexane (HPLC Grade; Fisher Scientific, Nepean, ON, Canada).

Extraction of Lipids

Prior to extraction, moisture in the tissues was removed. For adipocere samples, 20 mg of tissue was weighed out and lyophilized (Flexi-Dry™ MP Freezing Dryer, SP Scientific, Stone Ridge, NY) overnight. The dried tissue was then ground into a fine powder. The resulting powder was transferred into a glass vial with a polytetrafluoroethylene (PTFE) cap. Three milliliters hexane was added and mixed with the powder. At this point, heptadecanoic acid (2 mg/mL, 200 μL) was added to the mixture as an internal standard.

The sample was then sonicated for three 30-min sessions and centrifuged (Sorvall GLC-1 General Laboratory Centrifuge, Thermo Scientific, Waltham, MA) for 45 min at 1000×*g*. This procedure was then repeated two more times, resulting in a total volume of approximately 9 mL of extractant. The supernatant was concentrated by rotary evaporator (Heating Bath B490, Buchi Laboratory Equipment, Flawil, Switzerland) until about 1 mL of solvent was left. The remaining solvent was aspirated to dryness using a nitrogen stream. The dried sample was then redissolved in 1-mL hexane.

Derivatization of Individual Fatty Acids

FFAs contained in the 1-mL hexane were converted to their trimethylsilyl (TMS) derivatives by BSTFA with 1% TMCS.

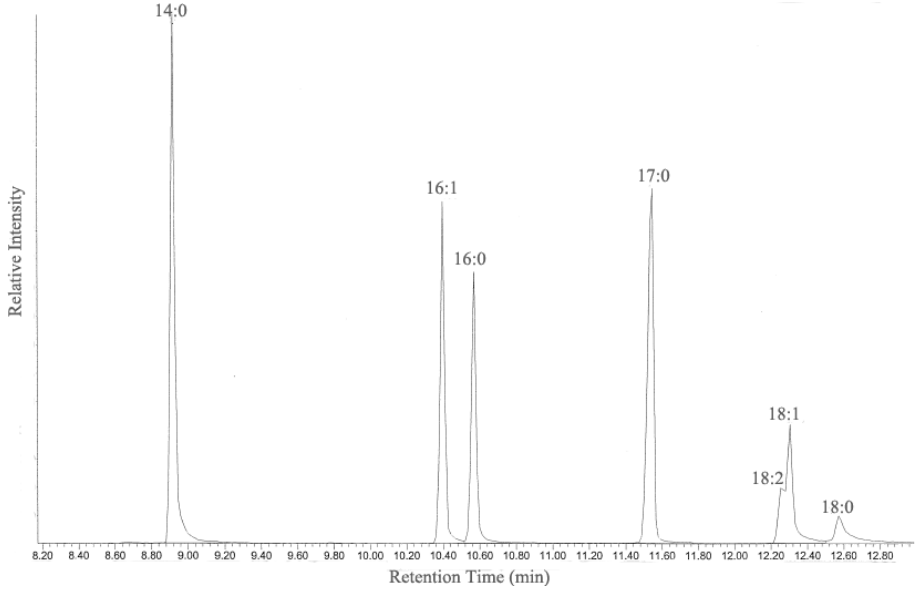

FIG. 1—*Partial gas chromatograms of trimethylsilyl derivatives of the standard mixture of lipids (500 μg/mL). The numerical notation associated to each peak shows the numbers of carbons and the number of double bonds, respectively, and the identity of the compounds are: 14:0-myristic acid, 16:1-palmitoleic acid, 16:0-palmitic acid, 17:0-heptadecanoic acid (internal standard), 18:2-linoleic acid, 18:1-oleic acid, 18:0-stearic acid.*

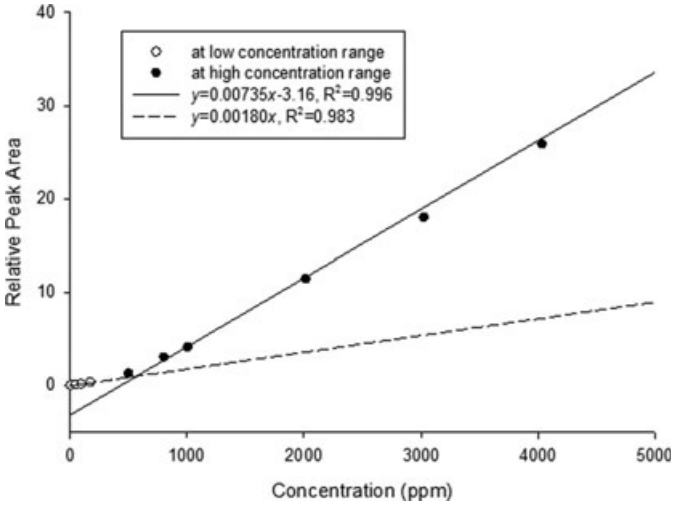

FIG. 2—*Relationship between relative peak area ($A_{Myristic}/A_{IS}$) and the concentration of myristic acid: (a) concentration from 500 to 5000 ppm, (b) concentration from 10 to 200 ppm.*

Excess silylation reagent was added (200 μL), and the vials were tightly capped with PTFE-sealed screw cap lids. The vials were then placed in a water bath at 60°C for 30 min. The TMS esters formed during this procedure were injected into the GC/MS for analysis.

Fatty Acid Analysis

The TMS fatty acid derivatives were analyzed by an Agilent 6890 Series GC coupled to an Agilent 5973 Network mass spectrometer. The analysis was monitored in total ion scan mode, and the fatty acids known to be present in adipocere were identified. The saturated fatty acids considered were myristic acid, palmitic acid, and stearic acid. The unsaturated fatty acids, palmitoleic acid, oleic acid, and linoleic acid, were also considered because of their possible presence in low concentrations. Peaks relating to the TMS esters of fatty acids were identified by comparison of their retention times and mass spectra with standards. The optimized conditions for the operation of the GC/MS system are listed in Table 1.

Results and Discussion

Calibration

A standard mixture of lipids (oleic acid, linoleic acid, palmitoleic acid, stearic acid, palmitic acid, and myristic acid) at a number of concentrations (10, 20, 50, 100, 200, 500, 800, 1000, 2000, 3000, 4000, and 5000 μg/mL) were dissolved in 1-mL hexane, and 200-μL heptadecanoic acid (2 mg/mL) was added as the internal standard. The TMS fatty acid derivatives in the mixture were efficiently separated under the optimized GC/MS conditions, as shown in Fig. 1.

The calibration curves are not linear throughout the large concentration range tested, and the concentrations of individual FFA in adipocere were significantly different. Therefore, the calibration curves to be used were constructed with a rather narrow range in the vicinity of the concentration of the fatty acids present in the sample. As shown in Fig. 2, while the slope of the calibration curve for larger concentrations of myristic acid is 0.0018, the slope of the calibration curve for smaller concentrations is 0.0073. As a result, it was necessary to create calibration curves for each of the individual fatty acids studied with concentrations relevant to the peak areas obtained from the tissue analysis.

Lyophilization

To increase the recovery of the FFAs in the tissues, it was necessary to grind the tissues into fine powders prior to the extraction of FFA with hexane. Because both the fresh muscle tissue and adipocere tissue contain moisture, the tissues were lyophilized first to dryness before grinding. Lyophilization also causes less damage to the material than other dehydration methods using higher temperatures (22). Additionally, the boiling points of fatty acids are relatively low, and lyophilization could reduce the loss of the fatty acids in the tissues.

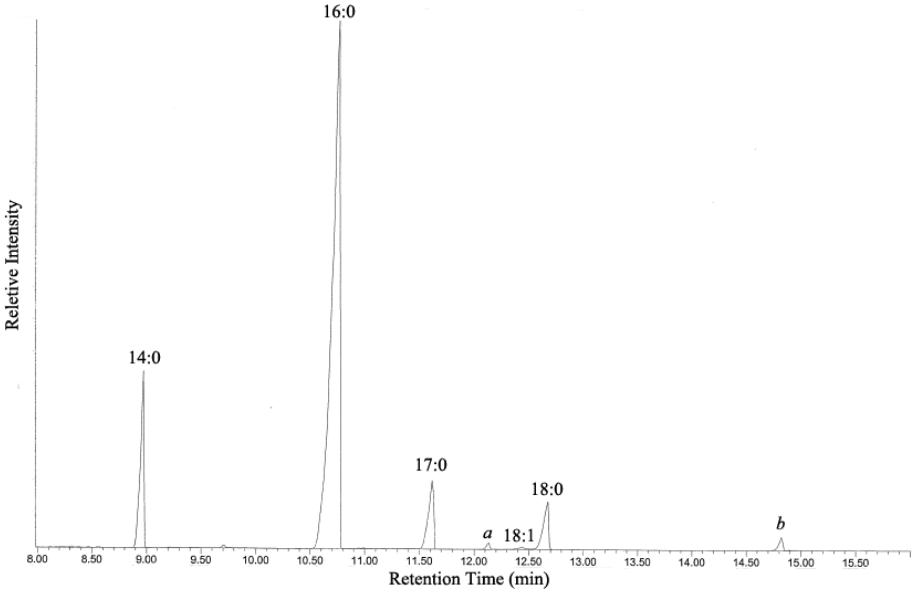

FIG. 3—*Gas chromatography/mass spectrometry chromatogram of fatty acid in Kwäday Dän Ts'ìnchí adipocere: 14:0-myristic acid, 16:0-palmitic acid, 17:0-heptadecanoic acid (internal standard), 18:1-oleic acid, 18:0-stearic acid, a-phytanoic acid, b-12-hydroxystearic acid.*

TABLE 2—*FFA concentration by weight (WT [μg/g]) and FFA relative composition (RC [%]) of fresh muscle tissue and adipocere of Kwäday Dän Ts'ìnchí (KDT).*

FFA		Fresh Muscle Tissue (Control)				KDT Sample			
		Biceps		Trapezius		Biceps		Trapezius	
FFA Name	Notation	WT	RC	WT	RC	WT	RC	WT	RC
Myristic acid	14:0	0	0.0	8	3.3	9405	9.0	9906	10.9
Palmitoleic acid	16:1	0	0.0	7	3.0	0	0.0	0	0.0
Palmitic acid	16:0	301	100.0	215	93.8	64434	61.4	54891	60.4
Oleic acid	18:1	0	0.0	0	0.0	8338	7.9	4433	4.9
Stearic acid	18:0	0	0.0	0	0.0	22778	21.7	21690	23.8

FFA, free fatty acids.

Application of the Method to the Samples

Figure 3 shows the GC/MS chromatogram of the total lipid extract (following trimethylsilylation) isolated from adipocere of Kwäday Dän Ts'ìnchí. The relative compositions of fatty acids in the tissues of Kwäday Dän Ts'ìnchí and the control subject are listed in Table 2. The concentrations of FFA in the control tissues, obtained from a recently deceased cadaver, are extremely low, and only palmitic acid could be detected with certainty. For the adipocere tissue of Kwäday Dän Ts'ìnchí, even-numbered, straight-chain saturated fatty acids were detected in abundance. Palmitic acid was the most abundant, with a relative composition of about 60% of the total FFA. This is because when the lipids were hydrolyzed by degradative anaerobes, they are converted from the neutral fat into FFA. This result is similar to those obtained from previous studies of adipocere (6,10,23). Some of the FFAs observed in this work have also been found in the bone and skin samples of Kwäday Dän Ts'ìnchí. Although the relative abundance varies somewhat depending on the types of tissue, the similarity between these results should be enough to suggest the presence of adipocere in the preservation processes (5).

The unsaturated fatty acids could be hydrogenated by bacterial enzymes to corresponding saturated fatty acids in humid and micro-aerobic conditions (14). As a result, the concentration of monounsaturated acids was low compared to the saturated fatty acids. Although oleic acid was observed, the peaks for palmitoleic acid and linoleic acid could not be seen in significant abundance.

Other Fatty Acid Components

As shown in Fig. 3, a few other peaks were seen in the chromotograms. The structures of these components were identified by their mass spectra to be 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) and 12-hydroxystearic acid. These fatty acids are also common constituents of adipocere (20). Because of the lack of appropriate standards, these components were not quantified in this study.

Conclusions

A simple and robust analytical method for the extraction, separation, and detection of FFAs from adipocere was developed and applied to the frozen human remains of Kwäday Dän Ts'ìnchí. Lyophilization of the tissues before extraction facilitated grinding of the tissue into fine particles to release the fatty acid components during the hexane extraction process. Using GC/MS, the relative fatty acid composition of adipocere in the Kwäday Dän Ts'ìnchí samples was determined. The results provided further evidence of adipocere in ancient tissues preserved in the glacier.

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