J Forensic Sci, January 2009, Vol. 54, No. 1
doi: 10.1111/j.1556-4029.2008.00911.x
Available online at: www.blackwell-synergy.com

Stephanie J. Notter,[1] *B.Sc.; Barbara H. Stuart,*[1] *Ph.D.; Rebecca Rowe,*[1] *B.Sc.; and Neil Langlois,*[2] *M.D.*

The Initial Changes of Fat Deposits During the Decomposition of Human and Pig Remains

ABSTRACT: The early stages of adipocere formation in both pig and human adipose tissue in aqueous environments have been investigated. The aims were to determine the short-term changes occurring to fat deposits during decomposition and to ascertain the suitability of pigs as models for human decomposition. Subcutaneous adipose tissue from both species after immersion in distilled water for up to six months was compared using Fourier transform infrared spectroscopy, gas chromatography-mass spectrometry and inductively coupled plasma-mass spectrometry. Changes associated with decomposition were observed, but no adipocere was formed during the initial month of decomposition for either tissue type. Early-stage adipocere formation in pig samples during later months was detected. The variable time courses for adipose tissue decomposition were attributed to differences in the distribution of total fatty acids between species. Variations in the amount of sodium, potassium, calcium, and magnesium were also detected between species. The study shows that differences in total fatty acid composition between species need to be considered when interpreting results from experimental decomposition studies using pigs as human body analogs.

KEYWORDS: forensic science, adipocere, adipose, gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), free fatty acids (FFA), inductively coupled plasma-mass spectrometry (ICP-MS)

The formation of adipocere is associated with the conversion of adipose tissue into a greyish-white, wax-like substance, which over time can become an armor-like solid mass. Adipocere is comprised of a mixture of saturated fatty acids that result from the late postmortem changes associated with the decomposition of adipose tissue in the body (1,2). In a forensic context, its presence becomes significant because of its ability to slow decomposition and, in some cases, preserve the remains.

The process of adipocere formation is initiated immediately after death by intrinsic lipases that hydrolyze adipose triglycerides to a mixture of free fatty acids. Given favorable environmental conditions, the unsaturated fatty acids undergo hydrogenation by bacterial enzymes to their saturated form. Hydrogenation of oleic, linoleic, and palmitoleic acids will yield stearic and palmitic acids, respectively. A single step β-oxidation may also occur (3).

Sodium and potassium released during autolysis or elements in the environment such as calcium from soil may react with cleaved fatty acids to form salts of fatty acids (4). This process is known as "hardening" and produces an insoluble product, which causes adipocere to have a more brittle quality (5).

The most abundant fatty acid found in adipocere is palmitic acid followed by stearic acid and then myristic acid (3,6,7). Other minor components of adipocere include triglycerides (6,8,9), hydroxy- and oxo fatty acids, such as 10-hydroxy- and 10-oxo stearic acid (10–13), and sodium, potassium, calcium, and magnesium salts of fatty acids (5,9).

The chemical composition and properties of adipocere has been reported in a number of studies (3,6,7,14–16). Speculation surrounding adipocere formation specifically in relation to the mechanisms involved and the initial changes to fat deposits is found in the literature (10,13). Most recently, experimental fieldwork using pigs as a model for differential decomposition of buried human remains highlights the need to investigate short-term changes in fat deposits (17).

The experiments reported in this paper were designed to investigate the changes in fat deposits in human and pig adipose tissue during the initial month of decomposition and thereby determine the chemical profile of early-stage adipocere formation. At present, contradictions exist in the literature as to when adipocere can first be detected. Some argue it may be detected after just 22 days postmortem (13,18), while others believe it may take months or even years depending upon the conditions under which the process takes place (19,20).

The suitability of pig tissue to model human adipocere formation was addressed in this study by submitting both types of tissue samples to the same conditions. The use of pigs (*Sus scrofa*) as body analogs is commonly encountered in human decomposition studies (13,17,21–26). This is mainly because of ethical restrictions, which preclude the use of human bodies in decomposition trials in countries including Australia. Pig adipocere has been studied since the late 18th century with Ruttan and Marshall (2) identifying similar fatty acid distributions between pig and human adipocere. Further studies have used pig cadavers to monitor the formation of adipocere in both soils (1,4,27–29) and water (13,20,24,30–32). However, very few reports address the suitability of pigs to mimic human adipocere formation. Although the fatty acid profile of human and pig adipose tissue is similar, pig adipose tissue triglycerides are dominated by saturated fatty acids (33) whereas human adipose triglycerides are dominated by unsaturated oleic, linoleic, and palmitic acids (34). Furthermore, unlike humans, pig adipose tissue triglycerides are greatly influenced by dietary intake (35). Therefore, a more detailed comparison between the formation of adipocere in pigs and humans is required to determine whether these structural differences affect the overall suitability of pigs as body analogs for human decomposition studies.

[1]Centre for Forensic Science, University of Technology, Sydney, PO Box 123, Broadway, NSW 2007, Australia.
[2]Department of Forensic Medicine, Westmead Hospital, PO Box 533, Wentworthville, NSW 2145, Australia.

Received 30 Dec. 2007; and in revised form 17 May 2008; accepted 21 June 2008.

This paper investigates adipocere formation in a model aquatic environment using pig and human adipose tissue. Those conditions known to favor adipocere formation—the complete submergence in a warm, anaerobic and aquatic environment, adequate skin and adipose tissue and sufficient bacteria—are used in this study. Infrared spectroscopy, gas chromatography-mass spectrometry (GC-MS) and inductively coupled plasma-mass spectrometry (ICP-MS) have been used to characterize the changes occurring to the fatty acid composition of adipose tissue during the first month of decomposition.

Materials and Methods

Sample Collection

Domesticated pig adipose tissue (*Sus scrofa*) was obtained from a local retail butcher. A 15 × 15 cm sample from the abdominal region containing some muscle and skin was used for each experiment. In accordance with the Human Tissue and Anatomy Legislation Amendment Act (NSW) 2003 ethical permission was obtained (ref: UTS HREC 2005-202) to use human tissue in the form of skin and subcutaneous fat resulting from elective plastic surgery after obtaining patient consent. For each experiment, a piece of human adipose tissue with similar dimensions to the pig adipose tissue was used.

Adipocere Formation

Tissue samples were completely submerged in deionized water in 5 L high density polyethylene airtight containers. The airtight containers ensured an anaerobic environment, which is known to favor adipocere formation (21). Three replicates for both the human and pig environments were prepared. A container with deionized water only was also prepared, as a control, to identify any fatty acids occurring because of the experimental set-up. The containers were held at 23°C for 6 months duration. Samples of tissue were collected every second day for the first month and then at 3 and 6 months. Samples were placed in sealed specimen containers, homogenized, and frozen at −18°C until further analysis. A sample of fresh pig and human adipose tissue was collected from the abdominal region to determine its fatty acid composition prior to decomposition and adipocere formation.

FTIR Spectroscopy

Two mg of adipose tissue was accurately weighed and ground together with 10 mg of powdered KBr using a mortar and pestle. The Spectra-Tech 3 mm microsampling cup (Spectra-Tech, Shelton, CT) containing the mixture was placed into a Nicolet diffuse reflectance infrared sampling accessory. The infrared spectra were recorded using a Nicolet Magna-IR 760 Fourier transform infrared spectrometer (ThermoFisher Scientific, Waltham, MA) equipped with a deuterated triglycine sulfate detector. One hundred and twenty-eight scans over the frequency range 4000–500/cm were recorded and Fourier transformed to give a resolution of 4/cm. Background spectra were recorded using 20 mg of powdered KBr.

Sample Preparation for GC-MS

As the adipose tissue contained small amounts of skin and connective tissue, it was necessary to extract the total lipids by a modified Folch et al. (36) method. Adipose tissue (1 g) was homogenized in a 20 mL solution of chloroform–methanol (2:1, v/v) and sonicated for 30 min. The sample was filtered and washed twice using 5 mL of the chloroform–methanol (2:1, v/v). Water was added in an amount of 0.2 times the volume of the filtered sample. It was then centrifuged (3500 rpm, 15 min) and the upper two layers were discarded. The lower layer was evaporated to dryness using a vacuum centrifuge for 15 min at 50°C. The lipid extract was dissolved in 3 mL of hexane for storing or directly used for the solid-phase extraction procedure.

Total Fatty Acid Analysis

Saponification of the adipose triglycerides was undertaken with 600 mg of adipose tissue, 20 mL of 20% NaOH solution, and 20 mL of ethanol. The mixture was refluxed for 30 min or until one layer remained. The solution was then cooled in an ice bath and 200 mL of water added. After cooling, the solution was acidified with 10% HCl solution and the resulting solid filtered. The solid was washed three times with cold water and allowed to dry. Five mg of the solid was then accurately weighed into a sterilized reacti-vial and dissolved in 1 mL of hexane with 200 μL of 10 μg/mL heptadecanoic acid added as an internal standard. The solution was derivatized using 250 μL of bis(trimethylsilyl) trifluoroacetamide at 60°C for 30 min after which an aliquot was removed for analysis by GC-MS.

Solid-Phase Extraction Procedure and Fatty Acid Analysis

Extraction of neutral lipids was performed using Bond Elut 100 mg aminopropyl disposable cartridge columns (Varian, Harbour City, CA) according to methodology previously reported (37). The trimethylsilyl (TMS) fatty acid derivatives were analyzed by an Agilent 6890 Series GC (Agilent, Santa Clara, CA) coupled to an Agilent 5973 Network mass spectrometer (Agilent). The GC-MS parameters are summarized in Table 1. The analysis was conducted in total ion scan mode and identified those fatty acids known to comprise adipocere. The saturated fatty acids considered were myristic, palmitic, stearic, and 10-hydroxy stearic acid. The unsaturated fatty acids, palmitoleic, oleic, and linoleic acid were also considered because of their occasional presence in low concentrations. Peaks relating to the TMS esters of fatty acids were

TABLE 1—*GC-MS operating parameters.*

Parameter	Condition
Column	DB-5MS (J&W Scientific, USA) fused silica capillary column (30 m × 0.25 mm × 0.25 μm, 5% phenyl 95% dimethylpolysiloxane)
Pressure	100 kPa
Carrier gas	Helium
Injection	
Volume	1 μL
Type	Split mode
Temperature	250°C
Split ratio	13.2:1
Split flow	20.0 mL/min
Oven	
Initial temperature	100°C for 2 min
Rate	10°C per min to 290°C held for 5 min
MS parameters	
Acquisition mode	Scan
Scan parameters	50–550 *m/z*
Solvent delay	5 min
Quadropole	
temperature	150°C
Source temperature	230°C

identified by comparison of their retention time and mass spectra against the NIST98 Mass Spectral Library. The relative response factor of the authentic standards and the unknown weight of the individual fatty acids were calculated relative to the internal standard heptadecanoic acid using known equations (38).

Elemental Analysis Using ICP-MS

Adipose samples were decomposed using acid digestion in combination with an oxidizing agent. A 5 mg sample was weighed into a polypropylene 10 mL screw-top vial to which 350 μL of each concentrated HNO_3 and HCl were added. This was heated on a hot plate until the emission of brown fumes ceased. Three hundred and fifty μL of H_2O_2 was added and the sample reheated until effervescence stopped. The solution was cooled and diluted to the graduation mark using high-purity deionized water. A 250 ppb internal standard containing ^{103}Rh and ^{45}Sc in 1% (vol/vol) HNO_3 was added to all samples during analysis through an external source connected to the ICP-MS.

Mixed calibration standards containing Na, Mg, Al, Si, K, Ca, Mn, Fe, Zn, Sr, and Pb were prepared from a 100 ppm stock solution via serial dilution to achieve final concentrations of 10 ppm, 1000, 500, 100, 10, and 1 ppb. Each standard was made up to 10 mL with high-purity deionized water and 350 μL of each concentrated HCl and HNO_3 added to achieve a similar matrix environment to the samples. Blank samples were prepared using high-purity deionized water and 350 μL of each concentrated HCl and HNO_3.

The analysis was carried out on an Agilent 7500ce ICP-MS Octapole Reaction System (Vic., Australia) with an ASX-510 Autosampler (Cetac, Omaha, NE) attached. The experimental parameters optimized are summarized in Table 2.

Statistical Analysis

Results are reported as the mean (Mn) percentage of all fatty acids for each component fatty acid type and where reported, the interval determined by subtracting and adding to the mean a quantity equal to two standard errors (SE) (Mn ± 2 SE). This interval provides a 95% confidence range of the true mean being estimated. Univariate and bivariate data analysis were performed using SPSS (SPSS, Inc., Chicago, IL). Where a comparison of means has been undertaken, either the Independent *t*-test or the Mann–Whitney test was applied depending upon the distribution of the data.

Results and Discussion

FTIR Analysis

An infrared spectrum of pig lipids obtained from adipose tissue prior to immersion is shown in Fig. 1. The spectrum shows the well-established characteristic bands of triglycerides (39), the major

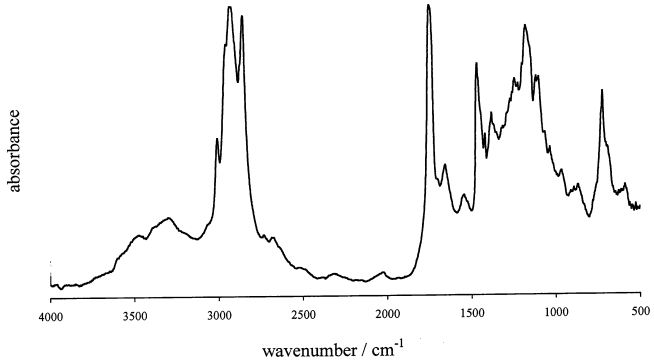

FIG. 1—*FTIR spectrum of pig adipose tissue prior to immersion.*

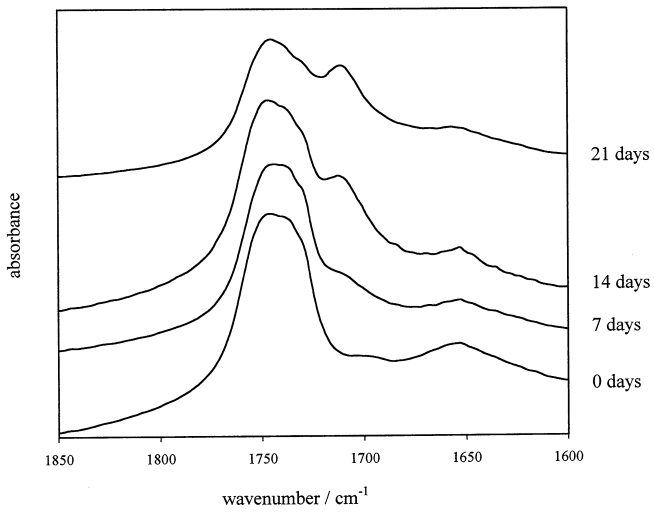

FIG. 2—*FTIR spectrum of the pig adipose tissue carbonyl region after immersion.*

lipid component of adipose tissue. A useful band for monitoring the breakdown of triglycerides into the component free fatty acids is the C=O stretching band in the 1750–1700/cm range. Figure 2 illustrates the carbonyl region of samples at 0, 7, 14, and 21 days after immersion, the time range where the notable changes to the spectra are observed. For the initial sample, the main peak observed at 1740/cm, with adjacent overlapping shoulders near 1730/cm. The band at 1740/cm is because of the carbonyl band of triglycerides, while the lower wavenumber bands near 1730/cm may be attributed to the presence of monoglycerides and diglycerides. Also in the spectrum of the sample at 0 days is a weak peak at 1710/cm that is because of the presence of free fatty acids. Changes to the relative intensities of the triglyceride and fatty acid C=O stretching bands as immersion time increases to 21 days are

TABLE 2—*Instrumental conditions and data acquisition parameters for ICP-MS.*

Parameter	Value	Parameter	Value
RF power	1550 W	Sampler/skimmer cone	Nickel
Sampling depth	7 mm		
Plasma gas	Argon	Sample uptake rate	0.80 rps
Auxiliary gas	0.45 L/min	Acquisition mode	Spectrum analysis (multi tune)
Carrier gas	0.7 L/min	Points per mass	1
Nebulizer	Micro-concentric	Dwell time	10 ms
Spray chamber	Glass, double pass	Number of replicates	5
Collision/reaction cell	Helium	Total acquisition time	66.7 sec

observed in Fig. 2. The free fatty acid band at 1710/cm increases in intensity, indicating a progressive breakdown of the triglycerides during this time period. Fatty acids also show a very broad carboxyl O-H stretching band in the range 3200–3400/cm. The O-H stretching band appears and increases in intensity in the spectra of the pig samples with increasing immersion time, correlating with the changes observed for the 1710/cm band.

The infrared spectrum of the human lipid sample taken prior to immersion is shown in Fig. 3. This spectrum is similar in appearance to that of the pig lipid sample prior to immersion shown in Fig. 1. The human samples show a main peak at 1740/cm similar to that observed for the pig sample. No peaks at 1730/cm are observed for the spectra of the early samples, but there appears to be a 1730/cm band in the spectra at 11 and 21 days, indicating the appearance of monoglycerides and diglycerides at these times. As was observed for the pig spectra, there is an increase in the amount of free fatty acids with increasing immersion time, indicated by the increase in relative intensity of the 1710/cm band in the human spectra (Fig. 4). However, the formation of fatty acids appears to be occurring at an earlier stage for the human samples. There is evidence of change at 5 days for human lipids, compared with 7 days for the first notable change in the pig lipid samples. As was observed for the pig samples, the fatty acid O-H stretching band also increases with time.

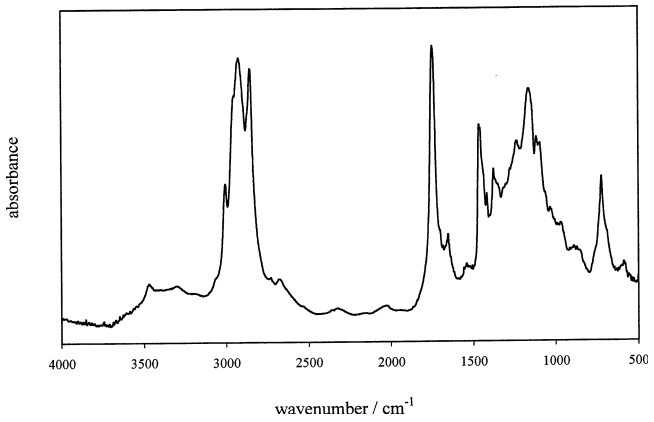

FIG. 3—*FTIR spectrum of human adipose tissue prior to immersion.*

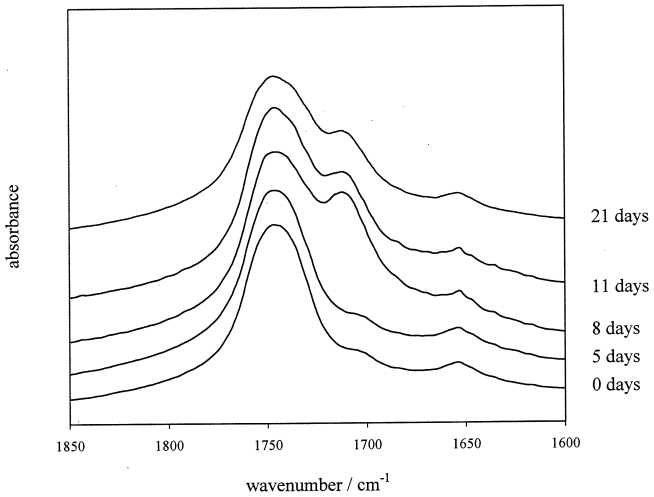

FIG. 4—*FTIR spectrum of the human adipose tissue carbonyl region after immersion.*

Total Fatty Acid Analysis

Table 3 describes discernible differences in the relative concentration of each fatty acid within both tissue samples. Oleic acid was the major fatty acid (18:1, Mn: pig—42.1%, human—43.9%) for both human and pig adipose tissue. High concentrations of palmitic and linoleic acid (16:0, Mn: pig—27.3%, human—38.2% and 18:2, Mn: pig—6.34%, human—13.4%, respectively) were also found in both species. These findings were consistent with the published literature (27,33,34,40–42). Differences in pig and human tissue were detected in the concentration of stearic acid, which was the third largest component in pig adipose tissue with *c.* 13% of the total fatty acids. The amounts of lauric, pentadecanoic, arachidonic, and eicosenoic acids were negligible (<1%). The unsaturated/saturated fatty acids (U/S) ratio also reflects larger amounts of saturated fatty acids in pigs compared with humans; however, unlike prior studies (41), these were not significantly distinguishable between species.

A statistical comparison of the means of each fatty acid between the species reveals significant differences in concentration for palmitic, linoleic, stearic, and lauric acids. Fatty acid concentrations are given in Table 3. Stearic acid concentration was higher in pig than human ($p < 0.05$). Palmitic, linoleic, and lauric acids, on the

TABLE 3—*Mean fatty acid concentrations (%) in human and pig adipose tissue.*

Fatty Acid	Human (n = 3)	Pig (n = 3)	H/P[†]
Total fatty acids—fresh tissue			
Lauric	0.20	0.02	10.0
12:0	(0.08–0.49)	(0.00)	
Myristic	2.48	0.85	2.92
14:0	(0.46–5.42)	(0.44–2.14)	
Pentadecanoic	0.17	Not detected	—
15:0	(0.03–0.30)		
Palmitoleic	3.73	2.42	1.54
16:1	(0.33–7.77)	(2.02–2.81)	
Palmitic	38.2	27.3	1.40
16:0	(30.2–46.2)	(21.8–32.8)	
Linoleic	13.4	6.34	2.11
18:2	(9.15–17.6)	(4.66–8.02)	
Oleic	43.9	42.1	1.04
18:1	(33.6–54.1)	(28.3–55.9)	
Stearic	4.55	12.9	0.35
18:0	(0.66–8.43)	(3.96–21.8)	
Arachidonic	0.05	0.06	0.83
20:4	(0.01–0.09)	(0.04–0.08)	
Eicosenoic	0.37	0.67	0.55
20:1	(0.00–0.74)	(0.22–1.12)	
U/S[*]	1.35	1.25	
Mean concentrations—decomposed tissue			
Myristic	1.75	1.01	1.73
14:0	(1.28–2.21)	(0.71–1.30)	
Palmitoleic	2.14	1.44	1.48
16:1	(1.61–2.68)	(0.73–2.15	
Palmitic	28.3	28.6	0.99
16:0	(26.5–30.1)	(23.3–33.8)	
Linoleic	7.74	5.98	1.29
18:2	(4.81–10.6)	(4.51–7.45)	
Oleic	52.3	41.6	1.26
18:1	(46.8–57.8)	(32.8–50.4)	
Stearic	6.29	15.1	0.42
18:0	(4.99–7.58)	(10.4–19.9)	
U/S[*]	1.66	1.13	

Each value represents the mean percentage and the interval of mean ± 2 SE.

*Total unsaturated/saturated fatty acids ratio.

[†]Human/Pig fatty acid ratio.

other hand, were significantly higher in concentration in humans than in pig adipose tissue ($p < 0.05$). It has been suggested that differences in enzymes and substrates involved in the digestion and reconstruction of triglycerides among species may account for the observed species-specific differences in fatty acid concentrations observed in this study (41). Furthermore, the exogenous origin of linoleic acid in both species reflects a higher dietary intake by humans compared with pigs (43).

Overall, the total fatty acid composition of pigs was significantly different from that of humans because of differences in fatty acid concentrations. Palmitic, stearic, oleic, and linoleic acids are dominant in the triglyceride structure of both humans and pigs, but differences in relative concentration between species may affect the overall chemical profile of adipose tissue decomposition.

Free Fatty Acid Analysis

Table 3 lists the relative concentrations of the free fatty acids identified in both the human and pig samples as determined by GC-MS. Fatty acids with the highest means of concentrations over the entire sampling period were oleic acid, palmitic acid, and stearic acid. Other fatty acids were present at concentration means between 0.09% and 8.50%. Trace fatty acids including arachidonic acid, eicosenoic acid, and 10-hydroxy stearic acid were excluded in this study for ease of comparison.

Box-whisker plots were used to compare fatty acid concentrations between the human and the pig samples. Figure 5 displays three fatty acids that had significant differences between the species. In the case of both myristic and oleic acids, human adipose tissue had significantly ($p < 0.005$) higher mean concentrations of each fatty acid over the entire sampling period compared with pig adipose tissue. This differed slightly from the total fatty acids, where oleic acid concentrations were comparatively similar. Stearic acid, on the other hand, remained significantly ($p < 0.001$) higher in pig adipose tissue. Although not statistically significant, human adipose tissue had a higher mean concentration of palmitoleic acid and a similar mean concentration of palmitic acid when compared with pig adipose tissue.

The higher ratio of U/S for humans is consistent with published results; however, this was not significantly distinguishable from the U/S ratio of pigs (41). Overall, higher levels of unsaturated fatty acids are found in humans compared with pigs during the initial decomposition of adipose tissue.

To determine the effect of these differences in fatty acid concentrations, the lipid profile was recorded for both species over a 6-month period. The resulting free fatty acid profiles are listed in Table 4. Initial inspection of the data revealed that adipose tissue samples had undergone only a slight degradation and had a chemical composition high in unsaturated fatty acids. In the initial month adipocere was not present in either tissue. Both tissue samples showed an increase in unsaturated fatty acids and a decrease in saturated fatty acids around the 5–8 day mark, which continued to progress for the majority of the first month. Interestingly, this change was reflected in the FTIR analysis, which noted the appearance of free fatty acids around this time. At 3 and 6 months pig adipose tissue samples showed a composition similar to early-stage adipocere formation. This was characterized by lower concentrations of unsaturated fatty acids and increased amounts of stearic and palmitic acids (1). Small amounts of 10-oxo- and 10-hydroxystearic acids were also detected, which is characteristic of adipocere (10,44). Human samples at the same time interval did not show these changes. As both tissue samples were allowed to decompose in identical situations, their observed differences are most likely because of the variations in composition of tissue. The higher levels of unsaturated fatty acids in fresh human tissue affect the conversion process, which is consistent with pig adipose tissue exhibiting more advanced decomposition. Alternately, the higher levels of saturated fatty acids in pig adipose tissue may be the result of

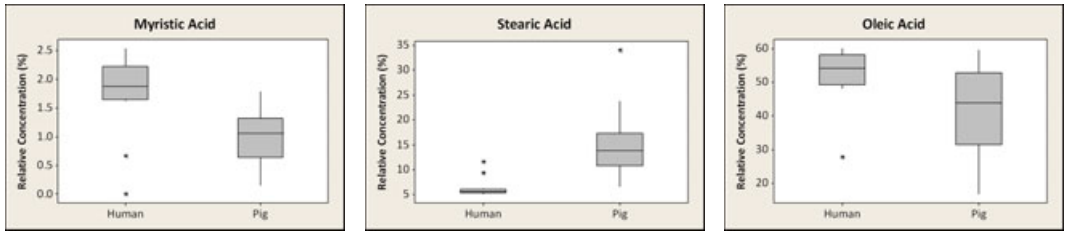

FIG. 5—*Box-whisker plots for three free fatty acids comparing differences in concentration between human and pig adipose tissue. Ordinates show concentration ranges of the compounds in percent of free fatty acids; see Tables 3 and 4.*

TABLE 4—*Change in relative concentration of fatty acids over the sampling period.*

	14:1 (%)		16:1 (%)		16:0 (%)		18:2 (%)		18:1 (%)		18:0 (%)	
Day	Human	Pig	Human	Pig	Human	Pig	Human	Pig	Human	Pig	Human	Pig
0	0.01	0.63	0.01	0.01	27.22	41.92	0.01	1.98	27.85	37.47	11.60	18.00
2	0.67	1.78	1.16	0.76	33.35	39.74	3.82	13.92	51.49	29.55	9.39	14.08
5	1.63	1.20	2.04	1.07	25.04	26.44	5.46	9.89	60.11	46.38	5.46	14.80
8	1.73	0.15	2.52	0.20	24.07	20.56	6.26	7.66	59.79	41.22	5.18	13.55
11	1.81	0.57	2.34	1.18	25.95	23.59	5.79	8.53	58.09	59.50	5.37	6.62
18	1.70	1.16	2.02	2.52	26.87	19.11	5.50	11.87	58.09	55.36	5.47	8.83
21	1.93	1.36	2.64	2.34	27.82	25.16	6.72	6.49	55.31	52.83	5.08	11.08
25	2.04	1.05	2.45	2.49	28.76	23.33	6.67	9.00	54.19	52.82	5.45	10.74
28	2.24	1.05	2.66	2.12	28.22	23.69	6.85	11.87	54.03	49.12	5.35	11.21
32	2.16	1.60	2.06	3.50	29.10	28.30	7.67	10.96	52.27	38.46	6.12	15.04
90	2.49	0.65	2.56	0.42	32.28	28.22	7.97	0.12	48.42	16.80	5.73	23.77
180	2.54	0.89	3.21	0.65	30.96	42.72	9.02	0.58	48.24	19.77	5.29	34.04

TABLE 5—*Relative percentage (%) of elements contained in adipose tissue during initial month of decomposition.*

	Na (%)		K (%)		Mg (%)		Ca (%)	
Day	Human (n = 3)	Pig (n = 3)	Human (n = 3)	Pig (n = 3)	Human (n = 3)	Pig (n = 3)	Human (n = 3)	Pig (n = 3)
0	76.45	8.48	18.55	81.20	1.63	4.98	1.93	0.94
2	62.50	5.08	17.40	61.90	4.07	6.13	6.95	9.55
5	50.45	4.82	21.20	62.15	6.53	10.80	6.28	4.99
8	48.10	4.21	22.90	64.85	4.62	9.33	9.41	3.50
11	44.80	3.64	26.35	56.40	5.34	8.21	10.75	4.48
18	40.80	3.46	20.30	55.95	5.69	10.65	18.05	5.05
21	38.55	2.80	24.05	59.70	6.24	9.26	16.80	6.93
25	40.20	3.60	26.50	60.50	4.90	11.10	14.10	7.04
28	44.05	3.56	23.40	54.30	5.24	21.90	10.55	6.20
30	50.40	1.31	23.85	32.65	5.12	57.00	6.20	5.06

higher levels of saturated fatty acids initially. Either way, these differences must be taken into account when using pigs as body analogs in adipose decomposition studies.

Elemental Analysis

Na, K, Ca, and Mg were detected at high levels in both samples by ICP-MS after acid digestion. Minor amounts of the trace elements Al, Zn, Pb, Si, and Mn were also detected. All elements are important in the physiological functions of various biological systems and are present in appreciable quantities in mammals (45). Relative concentrations of the major elements over the initial month are given in Table 5. Overall, there was little significant change over the initial month of decomposition in both tissue types; however, significant differences in concentration of Na and K ($p < 0.001$) were noted. Fresh pig adipose tissue contained large amounts of K (>80%) with <10% of the tissue containing Na. Conversely, fresh human adipose tissue contained more than 75% Na and <20% K. It is not clear why these elements differ so greatly in concentration between species; however, it does appear to be a distinguishable difference which may ultimately affect the type of salts formed in pig and human adipocere.

The change in concentration of elements in both species showed no clearly defined patterns. The general trend indicated a decrease in Na and K for both tissues except for K in human tissue, where the concentration increased slightly. This indicates a general diffusion of Na and K from the tissue cells into the surrounding environment as decomposition progresses. Furthermore, increases in both Mg and Ca suggest that fatty acids may be preferentially binding with these elements to form their respective salts. These trends are consistent in both pig and human tissue and indicate that they follow similar behavior, yet final concentrations at 30 days show varied compositions of these four elements in human and pig tissue. Human adipose tissue at 30 days contained mainly Na and K (50.40% and 23.85%, respectively), whereas pig adipose tissue was dominated by K and Mg (57.00% and 32.65%, respectively). As determined by GC-MS, no adipocere could be detected during the initial month and therefore the significance of these differences to adipocere formation in human versus pig adipose tissue could not be determined.

Conclusions

This study examined the chemical composition of pig and human adipose tissue degradation and the documented differences between these tissues. Results indicated that adipocere formation was not detected in the first month of decomposition in an ideal, experimental environment in either human or pig tissue. Differences in the rate of degradation between humans and pigs affected the appearance of adipocere in later months. This difference may be attributed to the total fatty acid composition, which varied between species. Higher levels of total saturated fatty acids in pigs showed adipose tissue decomposition to be more advanced and reflected fatty acid compositions consistent with earlier adipocere formation than in human tissue. This work indicates that although pig adipose tissue is similar to human adipose tissue and therefore suitable to mimic human decomposition, some margins of error exist when comparisons are being made between species.

Further studies will extend the period of decomposition to test for differences in the formation of adipocere and its associated salts in the longer term. The effect of environmental exposure will also be introduced in future work, which will greatly impact the types of fatty acid salts formed in adipose tissue.

Acknowledgments

The authors wish to thank Dr. Paul Curtin at Westmead Hospital, Sydney, for providing his assistance with the collection of tissue samples used in this study. We greatly appreciate his help and support in our research.

References

1. Forbes SL, Stuart BH, Dadour IR, Dent BB. A preliminary investigation of the stages of adipocere formation. J Forensic Sci 2004;49(3):566–74.
2. Ruttan RF, Marshall MJ. The composition of adipocere. J Biol Chem 1917;29:319–27.
3. Bereuter T, Lorbeer E, Reiter C, Seidler H, Unterdorfer H. Post-mortem alterations of human lipids—part I: evaluation of adipocere formation and mummification by desiccation. In: Spindler K, Wilfing H, Rastbichler-Zissernig E, Nedden zur D, Nothdurfter H, editors. Human mummies: a global survey of their status and the techniques of conservation. Wien: Springer-Verlag, 1996;265–75.
4. Forbes SL, Dent BB, Stuart BH. The effect of soil type on adipocere formation. Forensic Sci Int 2005;154(1):35–43.
5. Gill-King H. Chemical and ultrastructural aspects of decomposition. In: Haglund WD, Sorg MH, editors. Forensic taphonomy: the postmortem fate of human remains. Boca Raton: CRC Press, 1999;93–108.
6. Evershed RP. Chemical composition of a bog body adipocere. Archaeometry 1992;34(2):253–65.
7. Forbes SL, Stuart BH, Dent BB. The identification of adipocere in grave soils. Forensic Sci Int 2002;127:225–30.
8. Bereuter TL, Mikenda W, Reiter C. Iceman's mummification—implications from infrared spectroscopical and histological studies. Chem–Eur J 1997;3(7):1032–8.
9. Stuart BH, Forbes SL, Dent BB. Studies of adipocere using diffuse reflectance infrared spectroscopy. J Vib Spec 2000;24:233–42.

10. Takatori T. Investigations on the mechanism of adipocere formation and its relation to other biochemical reactions. Forensic Sci Int 1996;80:49–61.
11. Adachi J, Ueno Y, Miwa A, Asano M, Nishimura A, Tatsuno Y. Epicoprostanol found in adipocere from five human autopsies. Lipids 1997;32(11):1155–60.
12. Takatori T, Terazawa K, Nakano K, Matsumiya H. Identification of 10-hydroxy-12-octadecenoic acid in adipocere. Forensic Sci Int 1983;3:117–22.
13. Yan F, McNally R, Kontanis EJ, Sadik OA. Preliminary quantitative investigation of postmortem adipocere formation. J Forensic Sci 2001;46(3):609–14.
14. Fiedler S, Graw M. Decomposition of buried corpses, with special reference to the formation of adipocere. Naturwissenschaften 2003;90:291–300.
15. Kahana T, Almog J, Levy J, Shmeltzer E, Spier Y, Hiss J. Marine taphonomy—adipocere formation in a series of bodies recovered from a single shipwreck. J Forensic Sci 1999;44(5):897–501.
16. Takatori T. The mechanism of human adipocere formation. Leg Med 2001;3(4):193–204.
17. Wilson AS, Janaway RC, Holland AD, Dodson HI, Baran E, Pollard AM, et al. Modelling the buried human body environment in upland climes using three contrasting field sites. Forensic Sci Int 2007; 169(1):6–18.
18. Simonsen J. Early formation of adipocere in temperate climate. Med Sci Law 1977;17(1):53–5.
19. Dix J, Graham M. Time of death, decompostion and identification: an atlas. Boca Raton: CRC Press, 2000.
20. Mant AK, Furbank R. Adipocere—a review. J Forensic Med 1957;4:18–35.
21. Forbes SL. An investigation of the factors affecting the formation of adipocere in grave soils. [Dissertation]. Sydney: University of Technology, Sydney, 2003.
22. Hobischak NR, Anderson GS. Time of submergence using aquatic invertabrae succession and decompositional changes. J Forensic Sci 2002;47(1):142–51.
23. Hopkins DW, Wiltshire PEJ, Turner BD. Microbial characteristics of soils from graves: an investigation at the interface of soil microbiology and forensic science. Appl Soil Ecol 2000;14:283–8.
24. Mellen PFM, Lowry MA, Micozzi MS. Experimental observations on adipocere formation. J Forensic Sci 1993;38:91–3.
25. Micozzi MS. Experimental study of postmortem change under field conditions: effects of freezing, thawing, and mechanical injury. J Forensic Sci 1986;31(3):953–61.
26. Stuart BH, Craft L, Forbes SL, Dent BB. Studies of adipocere using attenuated total reflectance infrared spectroscopy. Forensic Sci Med Pathol 2005;1(3):197–201.
27. Forbes SL, Stuart BH, Dent BB. The effect of the method of burial on adipocere formation. Forensic Sci Int 2005;154(1):44–52.
28. Forbes SL, Stuart BH, Dent BB, Mulcahy SF. Characterization of adipocere formation in animal species. J Forensic Sci 2005;50(3):633–40.
29. Morgan ED, Cornford C, Pollock DRJ, Isaacson P. Transformation of fatty material buried in soil. Sci Archaeol 1973;10:9–10.
30. Gotouda H, Takatori T, Terazawa K, Mizukami K, Nagao M, Tarao H. The mechanism of experimental adipocere formation: hydration and dehydrogenation in microbial synthesis of hydroxy and oxo fatty acids. Forensic Sci Int 1988;1988(37):249–57.
31. Reh H, Haarhoff K, Vogt CD. The estimation of the time of death of corpses recovered from water (author's transl). Z Rechtsmed 1977;79(4):261–6.
32. Wasmund E. The formation of anabituminous adipocere under water. Neues Jahrb Mineral Geol 1935 Referate II:704.
33. Christie WW, Moore JH. Comparison of the structures of triglycerides from various pig tissues. Biochim Biophys Acta 1970;210(1):46–56.
34. Hirsch J, Farquhar JW, Ahrens EH Jr, Peterson ML, Stoffel W. Studies of adipose tissue in man. A microtechnique for sampling and analysis. Am J Clin Nutr 1960;8:499–511.
35. Christie WW, Moore JH. Effect of dietary copper on the structure and physical properties of adipose tissue triglycerides in pigs. Lipids 1969;4(5):345–9.
36. Folch J, Lees M, Sloane–Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
37. Notter SJ, Stuart BH, Dent BB, Keegan J. Solid-phase extraction in combination with GC/MS for the quantification of free fatty acids in adipocere. Eur J Lipid Sci Technol 2008;110:73–80.
38. Vane CH, Trick JK. Evidence of adipocere in a burial pit from the foot and mouth epidemic of 1967 using gas chromatography-mass spectrometry. Forensic Sci Int 2005;154(1):19–23.
39. Guillén MD, Cabo N. Infrared spectroscopy in the study of edible oils and fats. J Sci Food Agric 1997;75:1–11.
40. Davenel A, Riaublanc A, Marchal P, Gandemer G. Quality of pig adipose tissue: relationship between solid fat content and lipid composition. Meat Science 1999;51:73–9.
41. Kagawa M, Matsubara K, Kimura K, Shiono H, Fukui Y. Species identification by the positional analysis of fatty acid composition in triacylglyceride of adipose and bone tissues. Forensic Sci Int 1996;79:215–26.
42. Monziols M, Bonneau M, Davenel A, Kouba M. Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. Meat Science 2007;76:54–60.
43. Ruiz-Gutierrez V, Montero E, Villar J. Determination of fatty acid and triacylglycerol composition of human adipose tissue. J Chromatogr B 1992;581:171–8.
44. Takatori T, Yamaoka A. The mechanism of adipocere formation. 1. Identification and chemical properties of hydroxy fatty acids in adipocere. J Forensic Sci 1977;9:63–73.
45. Haraguchi H. Metallomics as intergrated biometal science. J Anal At Spectrom 2004;19:5–14.

Additional information and reprint requests:
Stephanie Notter, B.Sc.
PO Box 123
Broadway NSW 2007
Australia
E-mail: stephanie.j.notter@uts.edu.au