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

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Short title –Fat deposit changes in human and pig remains

ABSTRACT:

An examination of the early stages of adipocere formation in both pig and human adipose tissue in aqueous environments has been investigated. The aims were to determine at what stage this decomposition product first appears and to determine 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 (FTIR) spectroscopy, gas chromatography-mass spectrometry (GC-MS) and inductively coupled plasma-mass spectrometry (ICP-MS). 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 analogues.

KEY WORDS: 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 the soft tissue of a body into a grayish-white, wax-like substance, which over time can become an armour-like solid mass. Adipocere is known to comprise of a mixture of saturated fatty acids, which result from the late post-mortem changes associated with the decomposition of adipose tissue in the body [1, 2]. In a forensic context, its presence becomes significant due to 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, which hydrolyse adipose tissue triglycerides, via saponification, to a mixture of fatty acids. Given favourable environmental conditions, the unsaturated fatty acids may then undergo hydrogenation by bacterial enzymes to their saturated form. Hydrogenation of oleic, linoleic and palmitoleic acids will yield stearic, oleic and palmitic acids, respectively. Unsaturated fatty acids will also undergo a single step β-oxidation during the hydrogenation process. As palmitic acid is the major component of decomposed adipose tissue, a β-oxidation of stearic acid is assumed [3].

During decomposition cells undergoing autolysis release large amounts of Na and K into the tissues, which may attach to cleaved fatty acids to form salts of fatty acids. Additionally, sodium and potassium salts of fatty acids may be displaced by Mg and Ca ions to form new salts. This process is known as “hardening” and produces an insoluble product, which causes adipocere to have a more brittle quality [4]. These elements are also found in the environment, sometimes at higher concentrations than in the body, and therefore may be exchanged or displaced in preference to those from the surrounding environment. Adipocere formed in soils high in Ca will contain mostly calcium salts of fatty acids [5].

The major constituents of adipocere have been identified as myristic, palmitic and stearic acid [6]. These saturated fatty acids are present in characteristic ratios with palmitic acid being the most abundant fatty acid, followed by stearic acid and then myristic acid [3, 6,

7]. The minor components include triglycerides [7-9], 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 [4, 9].

The characterisation of the chemical composition and properties of adipocere is a subject of continuous interest and has been reported in a number of studies [3, 6, 7, 14-16]. Despite the increasing literature concerning decomposition and adipocere, the complex interrelationship between the chemical and environmental factors is such that speculation surrounding its formation remains, specifically in relation to the mechanisms involved and the initial changes to fat deposits [10, 13]. Most recently, experimental fieldwork using pigs to examine differential decomposition of buried human remains has highlighted the need for further investigation into short-term changes occurring to fat deposits in decomposition studies of forensic interest [17].

The experiments reported in the paper were designed to investigate the changes in fat deposits in human and pig adipose tissue over a short-term period. The purpose was to demonstrate the transformation of fat deposits into adipocere during the initial month of decomposition and thereby determine whether adipocere could be identified at these early stages. At present, contradictions exist in the literature as to when adipocere can first be detected. Some argue it may be detected after just twenty-two days post-mortem [13, 18], whilst others believe it may take months even years depending upon the conditions under which the process takes place [19-21].

The suitability of pig tissue to model human adipocere formation was also addressed in this study by assessing both types of tissue samples simultaneously under the same conditions. The necessity for this research stems from a need for a more comprehensive investigation into the suitability of pigs as models for human decomposition. The use of pigs (*Sus Scrofa*) as body analogues is commonly encountered in human decomposition studies

[13, 17, 22-27]. This is mainly due to ethical restrictions, which preclude the use of human bodies in decomposition trials in countries including Australia. Furthermore, the ease of access and similarities in body weight and organ morphology have suggested that pigs are the next most reliable model [28]. 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, 5, 29-31] and water [13, 20, 25, 32-34]. However, very few reports address the suitability of pigs to mimic human adipocere formation. Although research has shown that pigs are suitable in cardiac, dental, skeletal and immunological studies [35], the assumption of similarity between human and pig adipose tissue remains speculative. Although the fatty acid profile of human and pig adipose tissue is similar, pig adipose tissue triglycerides have an unusual configuration where saturated fatty acids dominate the structure [36]. This differs from human adipose triglycerides, where oleic, linoleic and palmitic acid usually dominate [37]. Furthermore, unlike human adipose tissue, pig adipose tissue triglycerides are greatly influenced by dietary intake [38]. 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 analogues for human decomposition studies.

The present paper investigates adipocere formation in a model aquatic environment using pig and human adipose tissue. Those conditions known to favour 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 in this study to characterise the changes occurring to the fatty acid composition of adipose tissue during the first month of decomposition.

Materials and Methods

Sample Collection

The decomposed tissue samples analysed in this study were prepared from pig and human adipose tissue. Pig adipose tissue from domesticated pigs (*Sus Scrofa*) was obtained from a local retail butcher. A 15 cm x 15 cm piece of pig adipose tissue collected from the abdominal region still containing some muscle and skin was used for each experiment. In accordance with the Human Tissue and Anatomy Legislation Amendment Act (NSW) 2003 waste tissue was procured after patient consent and ethical clearance was obtained. For this study, waste tissue in the form of skin and subcutaneous fat resulting from elective plastic surgery was used following strict ethical guidelines. For each experiment, a piece of human adipose tissue with similar dimensions to the pig adipose tissue was used.

Adipocere formation

The study utilized 5 L high density polyethylene containers, which were airtight and contained suspended baskets to hold the adipose tissue. The airtight containers ensured an anaerobic environment, which is known to favour adipocere formation [22]. De-ionised water was added until the adipose tissue was completely submerged. Three replicates for both the human and pig environments were prepared. A container with de-ionised water only was also prepared, as a control, to identify any fatty acids occurring due to the experimental set-up. The containers were held at 23 ºC for 6 months duration. Samples of tissue were collected every second day for the first month and then at three and six months. Samples were placed in sealed specimen containers, homogenized and frozen at -18 ºC 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 3mm microsampling cup 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 equipped with a deuterated triglycine sulfate detector. One hundred and twenty-eight (128) scans over the frequency range 4000-500 cm^{-1} were recorded and Fourier transformed to give a resolution of 4 cm^{-1}. 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 method [39]. Adipose tissue (1g) was homogenized in a 20 ml solution of chloroform-methanol (2:1, v/v) and sonicated for 30 min. The sample was then 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 of the two layers discarded. The lower layer was evaporated to dryness using a vacuum centrifuge for 15 min at 50 ºC. The lipid

extract was dissolved in 3 ml of hexane for storing or directly used for the solid-phase extraction (SPE) procedure.

Total Fatty Acid Analysis

Saponification of the adipose triglycerides was undertaken with 600mg of adipose tissue, 20 ml of 20 % NaOH solution and 20 ml of ethanol. The mixture was refluxed for 30 mins 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 (BSTFA) at 60 ºC 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 100mg aminopropyl disposable cartridge columns. The cartridge column was conditioned by rinsing it twice with 2 ml of hexane using the Visiprep extraction manifold. Lipid extracts from adipose tissue samples were dissolved in 1 ml of hexane with 200 µl of 10 µg/ml heptadecanoic acid added as an internal standard and applied to the column under gravity. Residual solvent was removed under vacuum. Thereafter, the column was eluted with 2 ml of a chloroform-2-propanol mixture (2:1, v/v). This fraction contained the neutral lipids (NL, Fraction 1). Then, 2 ml of diethyl ether containing 2 % acetic acid was applied to elute the free fatty acids (FFA,

Fraction 2). Care was taken to ensure the column did not dry out between the elution steps. The flow rate was maintained at approximately 2-4 ml/min. Each collected fraction was dried using a vacuum centrifuge at 50 ºC for 15 min. The remaining extract was taken up in 1 ml of hexane.

Fraction 2 (FFA) obtained from the column was derivatized using BSTFA. To the tubes, excess BSTFA was added (250 µl) and then tightly capped with Teflon sealed screw-cap lids. The tubes were then placed in an oven for 30 min at 60 ºC.

The TMS fatty acid derivatives were analysed by an Agilent 6890 Series GC coupled to an Agilent 5973 Network mass spectrometer. The GC-MS parameters are summarized in Table 1. The analysis was conducted in total ion scan (TIC) 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 identified by comparison of their retention time (RT) and mass spectra against the NIST98 Mass Spectral Library. The relative response factor (RRF) 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 [40].

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 then heated on a hot plate until the evolution of brown fumes ceased. 350 µl of H_2O_2 was then added and the

sample reheated until effervescence stopped. The solution was cooled and diluted to the graduation mark using high-purity deionised 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 ppb, 500 ppb, 100 ppb, 10 ppb and 1 ppb. Each standard was made up to 10 ml with high-purity deionised 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 deionised 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 (Victoria, Australia) with an ASX-510 Autosampler (Cetac) attached. The experimental parameters optimized are summarised in Table 2.

Statistical analysis

Results are reported as the mean (*Mn*) percentage of all fatty acids for each component fatty acid type and the interval determined by subtracting and adding to the mean a quantity equal to 2 SE ($Mn \pm 2$ SE). This interval provides a 95% confidence range of the true mean being estimated. Basic statistical calculations (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 Figure 1. The spectrum shows the well-established characteristic bands of triglycerides [41], the major 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^{-1} 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^{-1}, with an adjacent overlapping peak centred near 1730 cm^{-1}. Both peaks can be associated with triglycerides and the appearance of separate peaks at these wavenumbers has been previously reported and may reflect differences in the degrees of hydration of and/or hydrogen bonding to the ester carbonyl group [42]. Also in the spectrum of the sample at 0 days is a weak peak at 1710 cm^{-1} that is due to the presence of 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 observed in Figure 2. The free fatty acid band at 1710 cm^{-1} 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 3200-2400 cm^{-1}. 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^{-1} band.

The infrared spectrum of the human lipid sample taken prior to immersion is shown in Figure 3. This spectrum is similar in appearance to that of the pig lipid sample prior to immersion shown in Figure 1. However, on closer examination of the carbonyl region of the human spectrum reveals a difference to the initial samples between species (Figure 4). The

human sample shows a main peak at 1740 cm^{-1} similar to the pig sample, but with no peak at 1730 cm^{-1}. The variation in triglyceride carbonyl peaks between species indicates a difference in the hydrogen bonding in the triglycerides present. As was observed for the pig samples, 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^{-1} band in the human spectra. 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 to 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.

Total Fatty Acid Analysis

Samples of fresh human and pig adipose tissue were analysed to determine the fatty acids present in the total lipids and, thereby, determine any discernible differences in the relative concentration of each fatty acid within both tissue samples. The relative percentages of the major fatty acids in the total lipids of both human and pig adipose tissue, as determined by GC-MS analysis, are listed in Table 3. The fatty acid compositions of the total lipids shows that 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. However, unlike human adipose tissue, stearic acid was the third largest component in pig adipose tissue with ca. 13% of the total fatty acids. The amounts of lauric, pentadecanoic, arachidonic and eicosenoic acids were negligible (< 1%). These findings are consistent with previous reports, which show that oleic, palmitic and linoleic acids account for more than 80 % of the total fatty acids in both human and pig

adipose tissue [29, 36, 37, 43-45]. The unsaturated/saturated fatty acids (U/S) ratio also reflects larger amounts of saturated fatty acids in pigs compared to humans, however unlike Kagawa *et al.* [44], these were not significantly distinguishable between species.

Although this suggests that human and pig adipose tissues are similar, there are some significant differences in the relative percentages of individual fatty acids. 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. Stearic acid concentration was higher in pig adipose tissue (18:0, Mn = 12.9%) than human adipose tissue (18:0, Mn = 4.55%, $t(4) = 3.69$, $p < .05$). Palmitic, linoleic and lauric acids, on the other hand, were significantly higher in concentration in human adipose tissue (16:0, Mn = 38.2%, 18:2, Mn = 13.4% and 12:0, Mn = 0.20%) than in pig adipose tissue (16:0, Mn = 27.3, $t(4) = -4.83$, $p < .05$, 18:2, Mn = 6.34%, $t(4) = 6.66$, $p < .05$ and 12:0, Mn = 0.02, $t(4) = -2.74$, $p < .05$). It has been suggested that differences in enzymes and substrates involved in the digestion and reconstruction of TG among species may account for the observed species-specific differences in fatty acid concentrations observed in this study [44]. Furthermore, the exogenous origin of linoleic acid in both species reflects a higher dietary intake by humans compared to pigs [46].

Overall, the total fatty acid composition of pigs was significantly different from that of humans due to differences in fatty acid concentrations. Palmitic, stearic, oleic and linoleic acids are dominant in the triglyceride structure of both humans and pigs, yet 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 (18:1, *Mn*: pig – 41.6%, human – 52.3%), palmitic acid (16:0, *Mn*: pig – 28.6%, human – 28.3%), and stearic acid (18:0, *Mn*: pig – 15.1%, human – 6.29%). Other fatty acids were present at concentration means between 0.09 and 8.50%. Trace fatty acids including arachidonic acid (20:4), eicosenoic acid (20:1) and 10-hydroxy stearic acid (18:0,10-OH) were excluded in this study for simple comparison.

Box-whisker plots were used to compare fatty acid concentrations between the human and pig samples. Figure 5 contains such plots for three fatty acids, which had significant differences between the species. In the case of both myristic and oleic acids, human adipose tissue had significantly higher mean concentrations of each fatty acid over the entire sampling period (14:0, *Mn* = 1.74%, 18:0, *Mn* = 52.3%) compared to pig adipose tissue (14:0, M*n* = 1.01%, U = 23.0, $p < .004$ and 18:0, *Mn* = 41.6%, U = 36.0, $p < .05$). This differed slightly to the total fatty acids, where oleic acid concentrations were comparatively similar. The shift to greater concentrations of oleic acid may reflect the accelerated conversion of linoleic acid to oleic acid in human. Stearic acid, on the other hand had, remained significantly higher in pig adipose tissue (*Mn* = 15.2%) when compared to human adipose tissue (*Mn* = 6.29%, U = 7.00, $p < .001$). 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 to pig adipose tissue.

Similar to the total fatty acid analysis, the increased ratio of U/S for humans compared to pigs is consistent with published results, however according to our findings was

not significantly distinguishable from the U/S ratio of pigs [44]. The overall consequence of differences in the distribution of free fatty acids between species is higher levels of unsaturated fatty acids in humans compared to 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 six-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. Comparison of the fatty acids during the initial month revealed similar compositions to the fresh adipose tissue samples indicating that adipocere was not present in either tissue. Interestingly though, both tissue samples showed an increase in unsaturated fatty acids and a decrease in saturated fatty acids around the 5 to 8 day mark, which continued to progress for the majority of the first month. This change appears to be reflected in the FTIR analysis, which noted the appearance of free fatty acids around this time. At three and six months pig adipose tissue samples did however show a composition similar to early-stage adipocere formation. This was characterised 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, 47]. Human samples at the same time interval failed to show similar changes with unsaturated fatty acids still actively increasing in concentration. No oxo- or hydroxy fatty acids were detected in the human samples either. As both tissue samples were allowed to decompose in identical situations, their differences in composition illustrate that the tissue type is most likely responsible for affecting the formation of adipocere and the rate of decomposition. The higher levels of unsaturated fatty acids in fresh human tissue compared to pig tissue appears to be affecting the conversion process, which is reflected by pig adipose

tissue being in a more advanced state of decomposition. Alternatively, the higher levels of saturated fatty acids in pig adipose tissue may give the appearance of a more advanced state of decomposition, yet is purely the result of higher levels of saturated fatty acids to begin with. Either way, these differences must be taken into account when using pigs as body analogues in adipose decomposition studies.

Elemental Analysis

Elements within the adipose tissue of human and pig samples were analysed using ICP-MS after acid digestion. The elements important in this investigation were Na, K, Ca and Mg, which were detected at high levels in both samples. Minor amounts of the trace elements Al, Zn, Pb, Si and Mg were also detected. All elements detected play important roles in the physiological functions of various biological systems and are present in appreciable quantities in mammals [48]. Relative concentrations of the major elements over the initial month are given in Table 5. Preliminary results from this study revealed little significant change over the initial month of decomposition in both tissue types; however a comparison between the tissues revealed significant differences in concentration of Na and K ($p < .001$). Fresh pig adipose tissue contained large amounts of K (> 80 %) with less than 10 % of the tissue containing Na. Conversely, fresh human adipose tissue contained more than 75 % Na and less than 20 % K. It is not clear why these elements differ so greatly in concentration between species, however is 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 behaviour, yet final concentrations at 30 days show varied compositions of these four elements in human and pig tissue. Human adipose tissue still contains mainly Na and K (50.40 % and 23.85 % respectively), whereas pig adipose tissue is 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 demonstrated by examination of chemical composition the changes associated with pig and human adipose triglyceride degradation and the differences between both types of tissues. Results obtained indicate that adipocere formation could not be detected in the first month of decomposition in an ideal, experimental environment. The source of the tissue however, did appear to account for differences in the rate of degradation and the appearance of adipocere in later months. This difference may be attributed to total fatty acid composition, which was determined to vary between species. The higher levels of total saturated fatty acids in pigs show adipose tissue decomposition to be in a more advanced state and reflect fatty acid compositions consistent with adipocere earlier 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 into this effect will determine whether there are any long-term consequences to the formation of adipocere and its associated salts. The importance of environmental exposure will also be introduced in future work, which will greatly impact the types of fatty acid salts formed in adipose tissue.

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TABLE 1 – *GC-MS Operating Parameters*

Parameter	**Condition**
Column	DB-5MS (J&W Scientific, USA) fused silica capillary column (30m x 0.25mm x 0.25µm, 5% Phenyl 95% dimethylpolysiloxane)
Pressure	100 kPa
Carrier Gas	Helium
Injection	
Volume	1 µl
Type	Split mode
Temperature	250ºC
Split ratio	13.2:1
Split flow	20.0 ml/min
Oven	
Initial temperature	100ºC for 2 min
Rate	10ºC per min to 290ºC held for 5 min
MS parameters	

Acquisition mode	Scan
Scan parameters	50 – 550 *m/z*
Solvent delay	5 min
Quadropole temperature	150ºC
Source temperature	230ºC

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^{-1}	Acquisition mode	Spectrum Analysis (Multi Tune)
Carrier gas	0.7 L min^{-1}	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

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.

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

Day	14:1 (%)		16:1 (%)		16:0 (%)		18:2 (%)		18:1 (%)		18:0 (%)	
	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*

Day	**Na (%)**		**K (%)**		**Mg (%)**		**Ca (%)**	
	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

Figure Legends

Figure 1. FTIR spectrum of pig adipose tissue prior to immersion.

Figure 2. FTIR spectrum of the pig adipose tissue carbonyl region after immersion.

Figure 3. FTIR spectrum of human adipose tissue prior to immersion.

Figure 4. FTIR spectrum of the human adipose tissue carbonyl region after immersion.

Figure 5. Box-whisker plots for three free fatty acids comparing differences in concentration between human and pig adipose tissue.