Review of adipocere formation on decomposing bodies

By

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A thesis submitted in fulfilment of the requirements for the degree of
Master of Forensic Science (Professional Practice)

in

The School of Veterinary and Life Sciences
Murdoch University

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Semester 1, 2017

Murdoch
UNIVERSITY

Declaration

I declare that this manuscript does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due references has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed: Jess Lawn

Dated: 15/07/2017

Acknowledgements

Firstly, I would like to thank Dr Paola A. Magni for her guidance and constructive feedback during the construction of this paper.

I would also like to thank my family for their constant support and encouragement throughout this process and beyond.

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Part One – Literature Review

Review of Adipocere Formation on Decomposing Bodies

Abstract

Decomposition is a process that occurs in a body after death involving the breakdown of organic matter. Decomposition is influenced by a range of factors that act together with the general outcome being the complete degradation of a body.

Adipocere formation is a disruption of the typical decomposition process with the final result being the preservation of remains, human or animal. Adipocere arises from the decomposition of adipose tissue within the body. Adipocere is composed mostly of saturated fatty acids with hydroxy fatty acids, oxo fatty acids and fatty acid salts formed as by-products. The mechanism of formation is not fully understood although several theories have been proposed. Adipocere is not an end product and will degrade under the right circumstances, mainly exposure to aerobic conditions and may also be consumed by macrofauna.

The environments adipocere has been found in have been studied to elucidate the many factors that may influence formation. Water, soil, and dry environments are all capable of producing conditions conducive to adipocere formation. The main factors known to promote formation in all environments are an anaerobic environment, sufficient adipose tissue, warm temperatures, a mildly alkaline pH, bacteria and moisture.

Adipocere may form partially within the soft tissues or they may turn completely into adipocere, preserving the internal organs and bones which may also form adipocere. Formation can occur in as little as a day or form over many years.

Studying adipocere formation is important in forensic science as it may aid forensic professionals in their casework in terms of post-mortem interval and cause/manner of death determination and comparison between similar, previously reported cases and a current case may be useful in aiding the process of the investigation.

Additional research is needed in the area of adipocere degradation as there is little literature on it. The determination of the post-mortem interval is complicated by adipocere formation and there is still not a reliable way to estimate it in bodies with adipocere formation.

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List of Abbreviations

ATR-IR	Attenuated Total Reflectance Infrared Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
PMI	Post-Mortem Interval
VOCs	Volatile Organic Compounds

1. Introduction

Generally, after death, a body will start the process of decomposition and will pass through the various stages of decomposition from fresh to remains (Payne, 1965). The rate of decomposition is affected by a range of factors, both environmental and intrinsic to the body (Pinheiro, 2006). The stage of decomposition that a body is in when discovered in a forensic context can be used in conjunction with other indicators to estimate the post-mortem interval (PMI). The PMI is an estimation of the time between the death of an individual to the discovery of the body. Estimation of the PMI is important in a forensic investigation as it may aid in the identification of a body in circumstances such as a missing persons case and corroborate or contradict the alibi of a suspect (Madea, 2016; Thali et al., 2011). This estimation is complicated by processes that cause decomposition to be altered, as occurs with adipocere formation (Vass, 2011).

The term 'adipocere' was first introduced by Fourcroy in 1789, although Browne, in 1656, may have been the first to have made a reference to it (Barnes, 1934). Adipocere is a substance that forms on decomposing remains through the decomposition of adipose tissue found within a body. It represents a disruption of the decomposition process, slowing it down or halting it up to hundreds or even thousands of years under stable conditions (Aufderheide, 2011).

Adipocere hinders grave reuse in countries where cemeteries carry out this practice. Graves are generally left for 15-25 years during which the body should undergo complete decomposition (Fiedler & Graw, 2003). This process is hindered by adipocere and a grave may be opened with the discovery of a partially or fully preserved body still inside. The study of adipocere is important in a forensic context due to this property of inhibiting decomposition

(Forbes, Stuart, Dadour & Dent, 2004). Adipocere causes issues with determining the post-mortem interval but it may aid investigations by allowing identification of an individual due to preserved facial or body features, aid in the determination of the cause and manner of death due to preserved injuries, inform investigators about the type of environment it has formed in, and aid investigators in predicting what condition to expect a body to be in which may aid in better recovery of remains (Christensen & Myers, 2011; Mohan Kumar, Monteiro, Bhagavath & Bakkannavar, 2009; Pinheiro, 2006; Ubelaker & Zarenko, 2011).

The study of adipocere is important in aiding forensic professionals in their work. Published case studies that involve adipocere may aid an investigator in a current case if the cases share similar features. It is also important to be aware of the various environments adipocere forms in, the factors that influence adipocere formation, and the timing of its formation as this may assist in the determination of the PMI.

Review of the literature on adipocere formation will provide an update on the present knowledge of the chemical composition of adipocere, the mechanism of its formation, the numerous factors affecting its formation, and its formation throughout the body. The construction of a table detailing case studies involving adipocere formation will also provide forensic professionals with a reference of cases that could be referred to in casework.

2. Chemistry of adipocere formation

There has been extensive research into the chemistry of adipocere formation, both regarding its chemical composition and the mechanism of formation. Both human and pig (*Sus scrofa*) samples have been used in this research due to restrictions in some countries on the use of human remains. Pigs were found suitable to be used as a human analogue, although there are differences in the composition of adipose tissue and formation of adipocere between the species (Notter, Stuart, Rowe & Langlois, 2009). This requires a certain margin of error to be taken into consideration in applying findings to human adipocere formation.

2.1. Analytical techniques for adipocere investigation

Throughout the study of adipocere, numerous techniques and methods have been developed with the aim of producing analytical methods that are simple, fast and reliable to effectively examine the chemical composition and in extension the mechanism of formation. Bereuter, Mikenda and Reiter (1997) used attenuated total reflectance infrared spectroscopy (ATR-IR) to analyse a tissue sample from Ötzi, the Tyrolean Iceman. ATR-IR is a simple and less destructive technique than gas chromatography-mass spectrometry (GC-MS), however, the sample used must be a film or solution (Stuart, Forbes, Dent & Hodgson, 2000). Stuart, Craft, Forbes and Dent (2005) also used ATR-IR and found that triglyceride and C=O stretching bands could be utilised to observe the stage of adipocere formation. Stuart et al. (2000) used diffuse reflectance infrared spectroscopy. This technique can detect structural isomers whereas GC-MS cannot. The technique also allowed the use of powdered or soil samples and sample preparation was simple. Forbes, Keegan, Stuart and Dent (2003) developed a quantitative method using GC-MS to detect trace adipocere in soil. Forbes et al. (2004) used Fourier

transform infrared spectroscopy (FTIR) alongside GC-MS. FTIR allowed the identification of triglycerides and calcium salts in the adipocere samples in contrast to GC-MS. Notter, Stuart, Dent and Keegan (2008) used solid-phase extraction with GC-MS to quantify free fatty acids. Algarra, Rodriguez-Borges and Esteves da Silva (2010) used a liquid chromatography-mass spectrometry method to detect free fatty acids in soil. Finally, Liu, Park, Monsalve and Chen (2010) used lyophilization to convert the tissue being analysed into a fine powder before being extracted with hexane. This increased the amount of free fatty acids recovered from the sample and the sample was then analysed using GC-MS.

2.2. Chemical composition of adipocere

Fourcroy (as cited in Ruttan & Marshall, 1917) originally described the composition of human adipocere taken from cemetery samples as consisting of "soap of ammonia and phosphate of lime". Much later, a study by Ruttan and Marshall (1917) of adipocere formed on an almost 50-year-old pig sample showed it was composed predominantly of palmitic acid followed by hydroxystearic acids, oleic acid, stearic acid, and calcium soaps.

As analytical techniques advanced, techniques such as GC-MS and various infrared spectroscopy techniques were used to study adipocere composition (Adachi et al., 1997; Stuart et al., 2005; Stuart et al., 2000; Takatori & Yamaoka, 1977a, 1977b). These studies confirmed some previous findings while several new components were reported.

In particular:

- The following components were confirmed: Saturated fatty acids - palmitic, myristic and stearic acid, and unsaturated fatty acids - oleic, linoleic and palmitoleic acid, along

with fatty acid soaps (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}) and triglycerides (Adachi et al., 1997, Forbes et al., 2004, Stuart et al., 2000, Takatori & Yamaoka, 1977b).

- The following components were newly reported: Takatori and Yamaoka (1977a) studied the hydroxy fatty acids that had previously been identified and confirmed the position of the hydroxyl groups. Two hydroxy acids and their structures were determined using GC-MS from 3 to 6-month-old human adipocere. The hydroxy acids were determined to be 10-hydroxyhexadecanoic acid (10-hydroxypalmitic acid) and 10-hydroxyoctadecanoic acid (10-hydroxystearic acid) and comprised around 2-30% of the total adipocere composition.

  The same authors also discovered a new component – oxo (or keto) fatty acids, which were thought to be produced from the oxidation of hydroxy fatty acids. The two oxo fatty acids were 10-oxohexadecanoic acid (10-oxopalmitic acid) and 10-oxo-octadecanoic acid (10-oxostearic acid), and together comprised around 1-2% of the total adipocere composition (Takatori & Yamaoka, 1977b).

  Other components that have been found in some adipocere samples are 10-hydroxy-12-octadecenoic acid and *cis*-12-octadecenoic acid, derivatives of linoleic acid (Nushida, Adachi, Takeuchi, Asano & Ueno, 2008; Takatori, Terazawa, Nakano & Matsumiya, 1983), and epicoprostanol, a reduced compound of cholesterol (Adachi et al., 1997). Takatori (2001) also found several fatty acids in tissue from a newborn – 9-chloro-10-methoxy palmitic (9-methoxy-10-chloro palmitic) and 9-chloro-10-methoxy stearic (9-methoxy-10-chloro stearic) acid. Analysis has also been conducted on the volatile organic compounds (VOCs) of adipocere which found 18 VOCs associated with the substance (Hoffman, Curran, Dulgerian, Stockham & Eckenrode, 2009).

The chemical composition of adipocere has been used to classify various stages of formation, according to the percentage of each component, and to develop theories as to the mechanism of formation.

2.3. Mechanism of adipocere formation

Several theories that have been proposed for the process of adipocere formation include the fat migration theory, the saponification theory and the hydrogenation theory (Bereuter, Lorbeer, Reiter, Seidler & Unterdorfer, 1996; Takatori & Yamaoka, 1977a).

- The *migration theory* states that fat spreads throughout the surrounding tissue as decomposition of adipose tissue occurs. Triglycerides contained within the fat are broken down and the resulting free fatty acids, e.g. oleic acid, and glycerol diffuse or separate out due to gravity.
- The *saponification theory* states that the main reaction of formation is the hydrolysis of fat into fatty acids and glycerol. Insoluble soaps of the fatty acids are then formed.
- The *hydrogenation theory* states that fatty acids are released and spread into adjacent tissues. Unsaturated fatty acids are the main component and are hydrogenated into saturated fatty acids.

All three theories explain some observations of adipocere formation but none of them explains every element of it. The saponification theory is not thought to play a significant role in formation as soaps generally make up only a partial component of adipocere, around 2-35% (Bereuter & Lorbeer et al., 1996; Takatori & Yamaoka, 1977a). The hydrogenation theory is more widely supported by research, although Bereuter, Reiter, Seidler and Platzer (1996) noted in their experiment with adipocere from the Tyrolean Iceman that the tissue was in a

high oxidational state while the theory requires a reductive state. Adachi et al. (1997) also noted that the presence of epicoprostanol in adipocere indicated that both oxidation and reduction had occurred in the formation process due to the nature of epicoprostanol production from cholesterol.

Adipocere is formed through the post-mortem decomposition of adipose tissue within a body. After death, there is a migration of micro-organisms from the intestines and respiratory system into surrounding tissue. Aerobic organisms cause tissue oxygen depletion and an anaerobic environment begins to develop, causing a decline in aerobes. Anaerobic organisms then flourish and aid in the development of adipocere (Evans, 1963).

Adipose tissue consists mainly of lipids, of which 90-99% are triglycerides that consist of a glycerol molecule attached to three fatty acid molecules (Dent, Forbes & Stuart, 2004). Unsaturated fatty acids make up approximately 65% of the fatty acid content of human adipose tissue. Oleic acid is the most abundant fatty acid (~45%) followed by palmitic acid (~20%), a saturated fatty acid, and linoleic acid (~15%) (Hodson, Skeaff & Fielding, 2008). The overall process of adipocere formation is shown in Figure 2.1. The main process of adipocere formation involves both hydrolysis of triglycerides into the saturated and unsaturated fatty acids described previously, and hydrogenation of the unsaturated fatty acids into saturated fatty acids (Forbes et al., 2004). The main components of adipocere are saturated fatty acids with palmitic acid the most abundant (Bereuter & Lorbeer et al., 1996). Lipases within cells initiate the hydrolysis of the triglycerides for a brief period after which bacterial enzymes facilitate more extensive hydrolysis. If there is a sufficient concentration of enzymes and water the process will continue until most or all triglycerides are depleted. Water may be derived from the environment or from the body itself, either within the fatty tissue or drawn from surrounding tissue by diffusion (Evans, 1963). The fatty acid soaps, hydroxy fatty acids

and oxo fatty acids observed in adipocere are also produced as by-products of the process of formation (Forbes et al., 2004).

Sodium ions in the interstitial fluid and potassium in cell water may interact with the fatty acids released from adipose tissue to form their respective fatty acid soaps (Forbes et al., 2004). According to Vass (2001), adipocere will be hard and crumbly if sodium soaps are formed, and soft with a paste-like consistency with potassium soap formation. The environment a body is placed in will also contain metal ions, such as calcium and magnesium, that can displace the sodium or potassium ions (Dent et al., 2004). Powers (2005) states the opposite characteristics, the soap being hard and crumbly if potassium soaps are formed and soft and paste-like if sodium salts are formed, but that if sodium is displaced by calcium it will produce a more brittle substance.

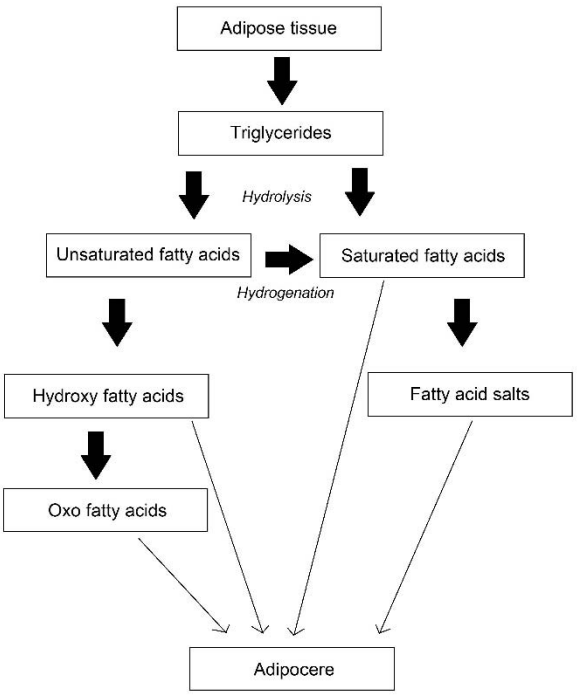

Figure 2.1 Flow diagram of the process of adipocere formation

Ruttan and Marshall (1917) noted that hydroxystearic acid was characteristic of adipocere and may be derived from oleic acid through hydration. Hydroxy acids are only detected in adipocere samples and not adipose tissue samples (Takatori & Yamaoka, 1977a). As noted earlier, oxo fatty acids were thought to be formed from hydroxy fatty acids as oxo fatty acids were not found in a 3-month old sample, while both were found in two 6-month old samples. A similar result was found by Adachi et al. (1997) in their study.

Both aerobic and anaerobic bacteria are thought to be involved in adipocere formation (Takatori, 1996). Many microorganisms including *Bacillus subtilis*, *Micrococcus luteus, Staphylococcus aureus*, some *Pseudomonas* species and *Clostridium perfringens* can convert oleic to hydroxy fatty acids. *Micrococcus luteus*, *Flavobacterium meningosepticum* and other *Pseudomonas* species can also convert hydroxy fatty acids to oxo fatty acids. This suggests that the bacteria have both cis-9-enoic acid hydrase and 10-hydroxy fatty acid dehydrogenase (Gotouda, Takatori, Terazawa, Nagao & Tarao, 1988; Takatori, Gotouda, Terazawa, Mizukami & Nagao, 1987). Fatty acids with a cis-9-unsaturation, as is the case with myristoleic, palmitoleic and oleic fatty acids, can be converted into both hydroxy and oxo fatty acids. Linoleic can only be converted to hydroxy fatty acids. Oxo fatty acids are not converted to hydroxy fatty acids by any of the bacteria.

Takatori and Yamaoka (1977b) and Takatori (1996) hypothesised that hydroxy and oxo fatty acids, along with fatty acid soaps, may play an important part in the formation and stabilisation of adipocere. The high melting point of the hydroxy fatty acids (78.5-79˚C) and oxo fatty acids (73.5-74˚C and 80-81˚C) were thought to be the reason behind their role in stabilisation, although oxo fatty acids only constitute a small percentage of the composition and do not appear in all samples of adipocere. Others suggested that palmitic and stearic acid will also contribute to stability as well as other fatty acids with high melting points (Serrulla

et al., 2016). Schoenen and Schoenen (2013), however, oppose the view that hydroxy fatty acids contribute to the stability of adipocere. They stated that studies have not taken microbial activity into account. The amount of oxygen necessary for complete decomposition by respiration is not available in environments conducive to adipocere and incomplete decomposition through fermentation occurs. Glycerol cleaved from triglycerides is degraded to carbon dioxide and water in respiration, while fermentation results in soluble alcoholic components that may wash away in the environment. Respiration degrades fatty acids through beta-oxidation into carbon dioxide and water but fermentation of fatty acids cannot occur and fatty acids are converted to polyhydroxy acids, a form of energy storage. These acids are insoluble in water and along with fat and long-chain fatty acids are not washed away. They remain where they originate in the body. The authors state that lack of oxygen is the true cause of adipocere formation and preservation.

Yan, McNally, Kontanis and Sadik (2001) produced a preliminary model for adipocere formation using pigs immersed in several water environments. They wanted to observe adipocere formation from the beginning as many previous studies had looked at later stage formation. Oleic acid was detected hours after each pig was immersed in water and the formation of adipocere followed the general trend of a decrease in oleic acid with a related increase in the level of hydroxystearic acid as time since immersion increased. The authors suggested that the ratio of oleic to hydroxystearic may be able to be used in improving time of death estimates, but that validation studies with human samples would need to be carried out as well as looking at factors that may influence the ratio. A theory as to the formation of hydroxystearic acid from oleic acid was also presented. Oleic acid was observed to decrease as hydroxystearic acid increased and this suggested that there was conversion of oleic acid into other derivatives, though the method of conversion is not clear. They suggested that

adipocere formation may be due to partial hydrogenation where some double bonds are saturated and some undergo stereomutation or bond migration. In the case of oleic acid, hydrogenation would produce stearic acid, stereomutation would produce [R-(Z)-12-hydroxycis-9-octadecanoic while hydration produces hydroxystearic acid and dehydrogenation of hydroxystearic results in oxostearic acid.

Another model of adipocere formation was proposed by Forbes et al. (2004) using pigs buried in soil. They classified adipocere into three stages of development – early, intermediate and advanced. Early stage formation indicates only minor tissue decomposition and the composition is close in similarity to adipose tissue but with a decreased unsaturated fatty acid content and increased saturated fatty acid content. Triglycerides are still detectable at this stage. Intermediate stage formation shows a further increase in palmitic acid and decrease in oleic acid with the addition of increased stearic acid content. Triglycerides are also still detectable at this stage. Advanced stage adipocere is indicated by palmitic acid comprising more than 50% of the total adipocere composition. Oleic acid is even more reduced and other unsaturated acids and triglycerides are no longer present. The stages are not distinct and one sample may fit into more than one stage, depending on which component is assessed. Another finding of the study was that classification into each stage was not dependent on the length of decomposition and other factors must enhance or inhibit formation. Stuart et al. (2000) also found similar results with a 26-month-old forensic sample having a comparable composition to a 27-year-old sample.

Notter and Stuart (2012) used the total percentage of saturated fatty acids to monitor the different stages. Early stage was 40-60%, intermediate was 70-90% and advanced was more than 90%.

The term for the formation of adipocere is sometimes referred to as saponification and is used interchangeably with the term 'adipocere formation', as is 'saponified tissue' and 'adipocere' (Ubelaker & Zarenko, 2011). As discussed above, saponification, the formation of soaps, does not constitute the entire process of adipocere formation but is still used by some to describe the process of hydrolysis and hydrogenation of fatty acids.

The intrinsic and extrinsic factors affecting adipocere formation, the formation of adipocere throughout the areas of the body and the timing of adipocere formation will be discussed in sections 3, 4 and 5.

2.4. Degradation of adipocere

Once adipocere is formed it may remain stable and unchanged for years, however, it is not an indestructible substance and is capable of being degraded. There are only a limited amount of reports on the degradation of adipocere and more research is needed in this area.

Pfeiffer, Milne and Stevenson (1998) examined microorganisms associated with adipocere and found Gram-negative bacteria - *Pseudomonas*, *Serratia*, *Alcaligenes*, *Enterobacter* and Gram-positive bacteria – *Bacillus*, *Nocardia*, *Cellulomonas*. It was observed that the Gram-positive bacteria hydrolysed the adipocere and that the resistance of adipocere to degradation was due to Gram-positive bacteria exclusion from the formation environment.

Fründ and Schoenen (2009) examined degradation of adipocere under various conditions. They determined that the half-life of adipocere is 11-82 years under anaerobic conditions while exposure to aerobic conditions resulted in a half-life of only 0.7-10 years. Burial in active soil was observed to have a half-life of 1.2-2.1 years. The conclusion reached was that exposure to aerobic conditions facilitated degradation.

Schoenen and Schoenen (2013) noted that degradation can only occur on the surface of the adipocere as there is a lack of oxygen within it. They cited another study in which the weight loss of adipocere samples was observed to be between 7.6% and 45.3% after a year. Adipocere degradation will be a prolonged process due to the insufficient exchange of oxygen in the environment.

Fiedler, Berns, Schwark, Woelk and Graw (2015) examined suspected degraded adipocere. Adipocere was observed to contain higher carbon and hydrogen levels while the degraded material had higher sulphur, nitrogen and oxygen. The authors postulated that in addition to aerobic degradation, poikiloaerobic conditions can degrade adipocere within burial environments. Poikiloaerobic conditions are conditions in which there is an alternating aerobic and anaerobic environment. Autoxidation of fatty acids, use of alternative electron acceptors to produce iron sulphide and the Maillard reaction, producing melanoidins were three reactions suggested to occur under these conditions.

2.4.1. Degradation of adipocere mediated by macrofauna

In addition to the degradative processes above, macrofauna are also associated with degradation of adipocere.

The fly genus *Piophila* is usually found on bodies that have been undergoing decomposition for around 3-6 months and in the presence of fatty acids. They may also appear at a later stage when adipocere formation is well marked. *Piophila* are found world-wide and are, in general, scavengers (Smith K. G. V., 1986). In a study looking at insect succession on pigs that were used to model human decomposition, *Piophila* species were found feeding on the adipocere and were observed to cause the adipocere to become foamy. They appeared from

90-92 days after death to 336 days after death. They were observed both on pigs that were left in the sun and pigs placed in the shade (Anderson, Hobischak, Beattie, & Samborski, 2002).

In China, a study conducted on beetle succession of pigs observed the species *Omosita colon* in association with adipocere and suggested that it may represent an important forensic indicator, especially in relation to adipocerous bodies (Lyu, Wan, Yang, Tang, & Xu, 2016).

2.5. Summary

Human adipose tissue is mainly comprised of unsaturated fatty acids while adipocere contains mostly saturated fatty acids due to the hydrolysis of triglycerides contained within the fat of adipose tissue and the subsequent hydrogenation of the unsaturated fatty acids released from the triglycerides. Fatty acid soaps, hydroxy fatty acids and oxo fatty acids are also present in adipocere as by-products. Any remaining triglycerides and unsaturated fatty acids, as well as epicoprostanol, cis-12-octadecenoic acid, 10-hydroxy-12-decenoic acid or 9-chloro-10-methoxy palmitic/stearic acid may also be present in some samples.

The formation of adipocere involves the presence of microorganisms that aid in the hydrolysis of fatty acids and transformation into hydroxy or oxo fatty acids.

Adipocere has been classified into several stages of development – early, intermediate and advanced. These stages aren't strictly defined and samples may fit in with more than one stage. The stage of formation is not dependent on the length of decomposition.

Persistence of adipocere is due to lack of oxygen. Adipocere is capable of degrading under aerobic conditions and may also be degraded under poikiloaerobic conditions. More research is needed in this area to determine additional factors in degradation.

The fly genus *Piophila* and beetle species *Omosita colon* have been associated with the degradation of adipocere.

3. Factors affecting adipocere formation

Bodies containing adipocere may be found in various water environments such as lakes, oceans and rivers, dry environments such as vaults, and soil environments. Each environment influences the formation of adipocere in differing ways in addition to other external and internal factors such as temperature, humidity, fat content and water content. These factors influence formation by enhancing or inhibiting adipocere formation and may also influence its composition. These factors will also influence the time of death estimates and at least some need to be considered when adipocere formation is present. The most agreed upon factors that are most conducive to adipocere formation in any environment are sufficient adipose tissue, mildly alkaline pH, moisture, anaerobic conditions, warm temperatures and the presence of bacteria (O'Brien & Kuehner, 2007; Ubelaker & Zarenko, 2011).

3.1. Water environments

Formation of adipocere may occur in a range of different water sources including seawater, rivers and lakes (Adachi et al., 1997; Heaton, Lagden, Moffatt & Simmons, 2010), bathtubs and wells, in warm, tropical waters and in cold seawater or melting glaciers (Bereuter & Lorbeer et al., 1996; Forbes, Wilson & Stuart, 2011; O'Brien & Kuehner, 2007). The factors that have been proposed to enhance and inhibit adipocere formation in water are listed in Table 3.1.

Table 3.1 Factors that inhibit and enhance adipocere formation in water environments

Factor	Enhance	Inhibit	Author (year)
Temperature	21˚C to 45˚C	Less than 21˚C	Forbes et al. (2011)
Clothing	Natural fibre material (wool, cotton) Synthetic fibre material (polyester, acrylic)	Absent	Notter and Stuart (2012); O'Brien and Kuehner (2007)
Skin slippage	Present	Absent	Widya, Moffatt and Simmons (2016)
Scavengers/ insects	Absent	Present	Forbes et al. (2011); Ueland, Breton and Forbes (2014)
Water type	River Distilled	Seawater Chlorine Saline Deionised	Stuart, Notter, Dent, Selvalatchmanan and Fu (2016); Yan et al. (2001)
Submersion	Complete submersion	Partial submersion	Notter et al. (2009); Ueland, Breton and Forbes (2014)
Bacteria	Gram-negative Gram-positive (early stages)	Lack of Gram-positive bacteria at early stages	O'Brien and Kuehner (2007); Ueland, Breton and Forbes (2014)

3.1.1. Temperature

The main influence on decomposition in an aquatic environment is the ambient water temperature (Heaton et al., 2010). Adipocere forms in a variety of different temperatures but at a variable rate (O'Brien & Kuehner, 2007). Formation of adipocere is enhanced by warm temperatures between 21˚C and 45˚C but adipocere may also form in cold water at a reduced rate. Cool temperatures between 7˚C and 16˚C promote adipocere formation into early or intermediate stages but if exposed to colder temperatures, it will not be allowed to develop into an advanced stage (Forbes et al., 2011).

Warm temperatures are thought to enhance formation because the activity of *Clostridium perfringens* is at its optimal between these values. Formation is thought to be able to proceed

even at temperatures below the optimum due to the bacterial enzymes released that will still be present and active after bacterial growth is arrested (Cotton, Aufderheide & Goldschmidt, 1987). The rate of formation in varying water temperatures has been reported as 12-18 months in 4˚C and 2-3 months in waters of 15˚C to 22˚C. Faster formation has been reported in 10˚C to 12˚C seawater after 38 days and 13˚C at 3 months (Forbes et al., 2011).

3.1.2. Submersion

Complete submersion in an aquatic environment is conducive to formation (Notter et al., 2009). It is likely to prevent contact with insects and delay decomposition due to cooler temperatures underwater. Insects and scavengers are more likely to have access to a floating body and the body may be swept along by currents, both of which inhibits adipocere formation (Dirkmaat, 2012; Ueland, Breton & Forbes, 2014). Forbes et al. (2011) found that the depth of submersion did not influence formation.

O'Brien and Kuehner (2007) placed three human bodies in water-filled pits, containing water from and underground pipeline, aiming to simulate field conditions. Liquid and tissue samples from each body were collected using a syringe with a needle and were analysed for fatty acids and microbes. Body 1 had open trauma, algal growth, and was submerged the entire experiment. Adipocere did not form on this body and it was determined that there was no C. *perfringens* on the body. Body 2 and 3 remained floating the entire experiment and adipocere formed on both bodies after 3 months. Signs of adipocere formation appeared around 6 weeks into the experiment within the water-level zone. As the two bodies that did exhibit adipocere formation remained floating, this showed that complete submersion, while favourable, was not necessary for formation and that absence of certain bacteria inhibits

formation as there was a lack of *C. perfringens* on the body that did not form adipocere. The temperature varied throughout the experiment and this indicated that stable temperatures are also not necessary for adipocere formation. The authors compiled a table of adipocere development stages. Early formation of adipocere was noted as 6-8 weeks, increased formation at 8-10 weeks, and advanced formation at 10-12 weeks.

3.1.3. Water type

The influence of water types has also been investigated. Yan et al. (2001) studied distilled, chlorinated and saline water to observe oleic acid degradation and hydroxystearic formation in the formation of adipocere. Distilled water showed the highest degradation rate while saline was the slowest. Conversion to hydroxystearic was most rapid in distilled water and the least in chlorine. Saturation of oleic degradation, the point where the rate of degradation did not increase further, was rapid in distilled and slow in saline.

Stuart, Notter, Dent, Selvalatchmanan and Fu (2016) found that seawater inhibits formation while river water enhances it. Chlorine was also studied and found to enhance formation, however, these results are complicated by the fact that there may have been a competing reaction of chlorohydrin formation that induced the appearance of enhanced adipocere formation. The different results were suggested to have developed due to the bacterial activity in each environment. Elemental analysis was also carried out which showed that only seawater significantly affects the elemental composition and the authors suggested that the differences could be used to determine in what type of environment a body has been immersed.

3.1.4. Bacteria

Ueland et al. (2014) investigated lake environments in relation to the effect of aquatic bacteria in early adipocere formation. This was carried out with pigs and involved collection of bacteria samples from both the water and tissue. It was discovered that the presence of Gram-positive bacteria is essential in early formation as they provide the means to break down the tissue and release fatty acids through lipolysis. Most Gram-negative bacteria are not capable of this. At later stages, Gram-negative bacteria exclude Gram-positive bacteria. Tissue submerged in deionised water, acting as the control, did not develop adipocere and bacterial concentrations of the lake tissue declined as adipocere formed.

3.1.5. Skin slippage

Direct exposure of adipose tissue to water via skin slippage was shown to promote adipocere formation in rabbits (Widya, Moffatt & Simmons, 2012). This was also seen by Ueland et al. (2014). Skin slippage is used as a variable in PMI estimations indicating that this is a potential factor to explore in improving estimates (Widya et al., 2012).

3.1.6. Clothing/material

The effect of clothing on formation is generally considered to enhance formation (O'Brien & Kuehner, 2007). Dix (1987), however, observed that clothes inhibited formation as areas with absent or loose clothing formed more advanced adipocere. Notter and Stuart (2012) studied several materials in which a body may commonly be covered or wrapped. Polyester, wool clothing, cotton clothing, wool carpet, and acrylic carpet were investigated. All samples

enhanced formation relative to the control sample. The wool and cotton materials made of natural fibre were better at enhancing formation than the synthetic polyester and acrylic carpet materials. Formation to an advanced stage of adipocere was faster in the natural fibre material. The wool carpet and the cotton clothing were the most efficient, followed by the wool clothing. The acrylic carpet enhanced formation better than polyester. The possible reasons given for the better formation with natural fibre material were the thickness of the material and water absorbency. Natural fibres are more absorbent than synthetic and the thicker wool carpet was better at enhancing formation than the wool clothing, possibly through absorbency or an insulating effect.

The effect of plastic on adipocere formation was unclear. Adipocere was expected to form more frequently than it was observed. Pakosh and Rodgers (2009) placed dismembered pig limbs into plastic bags and submerged them along with uncovered controls. The uncovered limbs lost soft tissue significantly more than the plastic enclosed, however, adipocere rarely formed within the plastic. This may have been due to low temperatures, low submersion time or low fat content. The limbs were preserved by the plastic but did not form adipocere.

3.2. Soil environments

Soil is composed of various minerals, salts, organic components and micro- and macroscopic organisms which all affect decomposition. When soil is removed from the ground and replaced during the burial of a body the nature of the soil changes. Compared to undisturbed soil, there is greater porosity and permeability of the soil. The passage of water and gases into the soil is also disrupted. The decomposition environment becomes anaerobic quite quickly and oxygen diffusion into the soil is thought to be blocked by gases from decomposition

diffusing out of the soil (Dent et al., 2004). Burial in soil also restricts insects and scavengers in contrast to bodies deposited on the soil surface (Carter, Yellowlees & Tibbett, 2007). These features create an environment conducive to adipocere formation. Factors that inhibit or enhance adipocere formation in soil environments are listed in Table 3.2.

3.2.1. Soil type

Many soil types have been tested and shown to promote adipocere formation. These include loam-rich, clay-rich, sandy loam, clay, clay-gravel, sterilised loamy sand, and loamy sand. Soils that have been found to inhibit formation are those with low clay content and high organic matter (Durães et al., 2010; Forbes, Dent & Stuart, 2005). In other cases, it is unclear whether soils such as sand and silty sand promote or inhibit formation. These are both well-draining soils and would not be expected to maintain much moisture (Schotsmans, Van de Voorde, De Winne & Wilson, 2011). Adipocere is not likely to form in a dry, desert sand environment but may form with burial in a sandy beach where there is constant moisture (Forbes et al., 2005). Fiedler and Graw (2003) considered the parent material the most important characteristic of soil whereas Durães et al. (2010) state that soil type is an indirect influence and the more crucial factor is the property of the soil, specifically the water content. Soils that retain moisture will generally promote adipocere formation. These soils would have poor drainage or be largely composed of fine particles (Fiedler & Graw, 2003). Strong, lateral water flow caused by soil such as clay also lead to high moisture (Fiedler et al., 2012).

3.2.2. Soil characteristics

Forbes, Stuart and Dent (2005a) studied the effects of soil pH, temperature, moisture and oxygen content. They found that mildly alkaline pH, warm temperatures, anaerobic conditions and both dry and wet soil promoted adipocere. Acidic pH, lime, cold temperatures and aerobic conditions inhibited it. The optimal pH was between 5 and 9 and temperature between 22-40˚C. At lower temperatures, formation will be inhibited while higher temperatures may promote desiccation and both inhibit bacterial growth. Excess moisture was not necessary but enhanced the rate of formation. Other researchers have found that acidic soils are conducive to formation. Schotsmans et al. (2011) found extensive adipocere on a body buried in a well-draining soil with a pH between 2.6 to 4. Biologically and microbially active soils will inhibit adipocere formation (Fiedler et al., 2009).

3.2.3. Method of burial

The method of burial can range from direct burial into the soil, burial within a coffin, burial within a mass grave and burial with or without clothing or material covering the body (Dent et al., 2004; Evans, 1963; Forbes, Stuart & Dent, 2005b).

Mass graves more commonly form adipocere than isolated, single graves (Evans, 1963). If the grave contains bodies piled on top of each other the layers tend to trap moisture and the middle of the grave will tend to contain bodies with the most adipocere formation. Bodies in mass graves that form only a single layer will exhibit decomposition closer to bodies that are buried in isolated, individual graves (Connor, 2009). Vane and Trick (2005) carried out research on a foot and mouth mass grave filled with pigs and cows. They found that there was a mixture of early and late stage adipocere. At a depth of 50-70cm adipocere was found

to be at an advanced stage along with samples of subcutaneous fat taken from the bodies. At 220cm, however, samples were only observed to be in the early stage of formation. This observation may be explained by mass graves containing variations in moisture resulting from infill that isolates various parts of the grave. This would result in a mixture of early and advanced stage adipocere.

Forbes et al. (2005b) found that direct burial in soil, clothing, and plastic and clothing promoted adipocere formation whereas burial in plastic and coffins were found to inhibit formation. Coffins inhibit formation due to slight aerobic conditions inside and will also prevent cation exchange compared to direct soil burial. Polyester clothing allowed cation exchange which is thought to have allowed hardening of the adipocere to take place.

A body that is buried at a depth of 90cm or below is generally safe from scavengers and will be exposed to a reduced number of insects and microorganisms (Fiedler & Graw, 2003). Evans (1963) observed that weather may have an influence and they observed that fog and misty conditions enhanced formation when they were present at the time of burial.

Oak, lead and zinc coffins enhance formation while pine and spruce cause rapid decomposition along with coffins lined with a straw bedding (Fiedler & Graw, 2003).

Table 3.2 Factors that enhance and inhibit adipocere formation in soil environment

Factor	Enhance	Inhibit	Author (year)
Temperature	Warm 20-40˚C	Cold More than 40˚C	Forbes et al. (2005a)
Scavengers	Absent	Present	Carter et al. (2007); Fiedler and Graw (2003)
Clothing/body coverings	Present Polyester	Absent Plastic coverings	Forbes et al. (2005b); Schotsmans et al. (2011)
Soil type	Loam-rich Clay-rich Sandy loam Loamy sand Clay Clay gravel Sand Sterilised Silty sand Poor draining Fine particles	High organic matter Gravel Active soil Low clay content	Durães et al. (2010); Fiedler and Graw (2003); Fiedler et al. (2009); Forbes et al. 2005
Moisture	High Excess Lateral water flow Fog and mist at time of burial	Low	Evans (1963); Fiedler et al. (2012); Forbes et al. (2005a)
Oxygen	Low, anaerobic	High, aerobic	Forbes et al. (2005a); Schotsmans et al. (2011)
Method of burial	Directly in soil Mass grave Oak, lead and zinc coffins Clothing with plastic Depth at or below 90cm	Pine and spruce coffins Straw bedding Wood shavings Lime Coffin	Dent et al. (2004); Evans (1963); Fiedler and Graw (2003) Forbes et al. (2005b); Green (2006); Schotsmans et al. (2011)
Soil pH	Mildly alkaline pH 5-9	Acidic Highly alkaline	Forbes et al. (2005a)

3.3. Dry environments

Development of adipocere was thought to require a damp or moist environment, however, cases of adipocere in dry environments have been reported. Adipocere formation may be found in combination with desiccation in this environment (Schotsmans et al., 2011). Research was carried out by Evans (1963) on bodies in dry vaults where over half of the bodies in each vault had formed extensive adipocere. This suggested that the internal water of the bodies was sufficient for formation and abundant moisture was not necessary.

Green (2006) also studied bodies contained in vaults consisting of an outer wooden shell and a sealed lead coffin with inner untreated wooden lining inside. Inside, the bodies were laid on wood shavings. In earth burials, wood shavings or sawdust enhance decomposition, however, the airtight condition of the coffins was assumed to produce sufficient conditions to promote adipocere.

Zimmermann, Laskay and Jackson (2008) described a case where suspected adipocere was found on a dry, indoor concrete surface. The body had been removed from the spot where it had been laying for several weeks and a stain had formed on the concrete surface. Conditions within the room were unclear but the conditions were thought to have been warm enough for bacterial activity as the body was quite decomposed when it was removed.

Nushida et al. (2008) described a case of a female that was sealed in a clothes box covered in plastic bags for 4 years. Adipocere formed extensively throughout the body, within the subcutaneous and the visceral fat.

Adipocere formation in environments has been described as typical or atypical (Nushida et al., 2008). Typical conditions were identified as water immersion, wet graves and damp vaults

while atypical conditions were dry conditions. The authors stated that adipocere forms more than 10 times slower in atypical conditions.

3.4. Summary

Adipocere formation is observed in water, soil and dry environments. Each environment will have varied factors and requirements for adipocere formation. Many factors influence adipocere formation but the most important for any environment are sufficient adipose tissue, mildly alkaline pH, moisture, anaerobic conditions, warm temperatures and the presence of bacteria. The influence of other factors is less clear and sometimes contradictory.

Adipocere formed in water is enhanced by temperatures between 21˚C and 45˚C, clothing and material made from natural fibres, the absence of scavengers and presence of bacteria, skin slippage, river environments and complete submersion. Inhibitors include cold temperatures, the absence of clothing and synthetic clothing or material, and scavengers.

Soil environments influence adipocere formation through factors such as soil pH, moisture, temperature, soil type and burial method. A slightly alkaline (pH 5-9), moist, anaerobic conditions with direct burial in the soil enhances formation while acidic, aerobic conditions, lime and plastic inhibit formation.

A dry environment is also conducive to adipocere formation as long as there is sufficient water contained within the tissues of the decomposing body.

A wide range of other factors in each environment play a role in decomposition and enhancing or inhibiting adipocere formation. When developing improved methods for estimating PMI, to get the most accurate estimate these would all need to be taken into account.

4. Timing and distribution of adipocere within the body

The general description of adipocere varies considerably. It may be yellow, greyish white, red, grey and grey-green and has a characteristic odour (Ubelaker & Zarenko, 2011; Pinheiro, 2006). It may form on a small area of the body or cover the body entirely (Kasuda et al., 2016). It was thought that only the subcutaneous tissues formed adipocere, however internal organs and bone marrow cavities can exhibit adipocere formation if adequate fat is present. Even in areas with low fat content, the pressure from putrefactive gases can cause liquefied fat to penetrate the tissue (Evans, 1963).

Just as formation varies with the environment a body is in, decomposition and adipocere formation vary between different bodies and even different areas of the same body. Bereuter and Lorbeer et al. (1996) cited a case of a man and woman in the same car submerged in a mountain lake. The man was skeletonised while the woman's soft tissue was preserved by adipocere. This was thought to be due to the different fat composition between men and women as women generally have a higher fat content than men. Ferreira and Cunha (2013) looked at 25 cases in a cemetery, all with a similar PMI of approximately 5 years but with different stages of decomposition. Desiccation and adipocere have also been shown to occur on the same body under dry conditions where there is little external water present. A male buried in a shallow grave with his right leg and arm and temporal bone exposed exhibited desiccation in the exposed areas and adipocere in the areas covered by soil (Schotsmans et al., 2011).

4.1. Timing of formation

Formation of adipocere is stated to be most commonly observed at three to six months with partial formation at six weeks and extensive formation taking at least a year (Bereuter & Lorbeer et al., 1996). It is generally reported that the initial development within cutaneous areas of the body occurs after a couple of months. Organs may remain preserved in their natural state even after 2 years. Muscles will start transformation a least 6 months after the initial transformation process begins and complete transformation is reported to take around 2 years (Thali et al., 2011).

There have been several cases in which formation has been very rapid. These have mostly been in very hot and humid environments in countries such as India.

Sikary and Murty (2015) discussed several cases in which this formation was observed in less than 2 days. Most of the bodies were located inside without air conditioning. These bodies had a roughly equivalent extent of formation between them. Several bodies were also recovered in water and showed a more variable extent of adipocere formation. There was no correlation between the age of each person and the formation of adipocere. Mohan Kumar et al. (2009), also in India, observed formation on a body within 3 days of submersion in a marshy pond. A male buried in gravel and shale with heavy rainfall before and during the period of burial formed adipocere in around 4 days (Powell, 1917).

A body is rarely observed to be completely converted into adipocere involving both the subcutaneous tissue and the internal organs. A recent case of complete transformation was found in a male after 7 years of immersion within a car at the bottom of a lake and was used to attempt to explain the process of complete adipocere formation (Kasuda et al., 2016). Submersion at the bottom of the lake with the car relatively undamaged was thought to have

created a restrictive environment with low pH. There is little or no water flow at the bottom of a lake and this is thought to have contributed to maintaining a low pH and may have inhibited formation of adipocere causing a slow conversion of the body. Absence of oxygen at the bottom of lakes creates desirable conditions for anaerobic bacterial growth. Oligotrophic conditions found in this environment also contribute to complete formation as well as the lack of scavengers.

Cotton et al. (1987) presented a similar case of extensive subcutaneous adipocere but without transformation of the organs. Two bodies were submerged in a vehicle in fresh water for 5 years. They were thought to have entered the water when the temperature of the water was at its highest point. The temperature subsequently dropped and this may have slowed down formation.

4.2. Subcutaneous tissue

Adipocere formation is initiated within the subcutaneous tissue and spreads outwards. The most common areas of formation are the cheeks, buttocks, abdomen and breasts. Adipocere is not likely to form on the hands or feet due to their relatively low fat content. Hands and feet tend to skeletonise and become disarticulated especially in water environments. An increase in the weight from the increased density due to formation of adipocere may be experienced. A general increase in the size of a body may also occur (Cascio & Teodoro, 2014; Fiedler & Graw, 2003). Adipocere is generally more common in women, newborn babies, obese and well-nourished bodies due to their higher fat composition (Byard, 2016). There has been some correlation between age and formation of adipocere but others have not observed this (Evans, 1963; Sikary & Murty, 2015; Fiedler & Graw, 2003).

Adipocere formation within the soft tissue may aid in preserving identifiable features of the body such as perimortem injuries and facial features (Gupta & Jain, 2011; Mohan Kumar et al., 2009). Toxicology may also be able to detect chemicals, such as toluene, within a body that has been converted to adipocere (Inoue et al., 1996; Tanaka, Sato & Kasai, 2016). Adipocere distribution throughout the subcutaneous tissue has been visualised using multislice computed tomography (Jackowski et al., 2005).

4.3. Organs

Organs may be found to have been preserved simply due to the anaerobic environment produced by a shell of adipocere developed over the body in the subcutaneous fat or they may themselves be converted into adipocere (Cotton et al., 1987). Preservation of organs depends on the level of putrefaction at which adipocere begins and organs that have been well preserved indicate that formation occurred closely after death in a suitable environment. Organs are commonly found in a dehydrated form when adipocere covers their surface (Schotsmans et al., 2011).

Different organs will have different rates of decomposition and some will be preserved better than others. The intestines and spleen are quickly putrefied followed by the brain. The heart is more resistant followed by the kidney, lungs and bladder. The prostate and uterus are very resistant to putrefaction (Pinheiro, 2006). Cheeks, eye sockets, chest, abdominal wall and buttocks commonly form adipocere. Adipocere is self-promoting due to inhibition of putrefaction, increasing acidity and dehydration, reducing growth and spread of putrefactive bacteria.

The brain is the organ that is least expected to be preserved by adipocere (O'Connor et al., 2011; Papageorgopoulou et al., 2011). Only two cases of adipocere formation in the brain have been confirmed. In other cases, chemical analysis of the brains was not carried out or it was unclear if it was carried out. Most cases of suspected adipocere formation in brains have been observed in archaeological or historical contexts with cold temperatures and acidic soil (O'Connor et al., 2011; Serrulla et al., 2016; Serrulla, Etxeberria, Herrasti, Cascallana & Olmo, 2017; Papageorgopoulou et al., 2011).

O'Connor et al. (2011) pointed out that lipids are a component in brain tissue but mostly in the form of phospholipids or glycolipids. Triglycerides and free fatty acids constitute only a tiny portion of total lipid content. They also noted that all the reported brains were shrunken, whereas adipocere usually increases the volume of the body

Some of the most well-preserved adipocere brains were those found in Spanish Civil War mass graves (Serulla et al., 2016; Serulla et al., 2017). The bodies were laid side by side and the bodies with adipocere were spread throughout the area. The gross morphology of the brains was present but could not be recognised histologically. It was proposed that the formation of adipocere in the brains was due to the gunshot wounds to the head which caused blood loss and inhibited brain decomposition. The climate at the time of burial was colder than usual with heavy rainfall which would lead to waterlogged soil. The wounds allowed water to come into contact with the brain and adipocere formed.

The other confirmed case of adipocere by Papageorgopoulou et al. (2011) involved the preservation of the left cerebral hemisphere of a 13th century infant. The main brain structures were well preserved and could be identified macroscopically and microscopically. The body was situated in a leather envelope within a wooden coffin buried in acidic, clay soil that contained fresh and salt water. Continuous water immersion inside the grave was

thought to have aided adipocere formation. The analysis carried out on this brain was only qualitative and it is difficult to determine to what extent adipocere conserved the brain. Adipocere may have only partially played a role in preservation. It is possible that a red-brown deposit on the surface may have been adipocere but it was not analysed (O'Connor et al., 2011).

Serrulla, Etxeberria and Herrasti (2015) found that within the same Spanish Civil War mass grave as described above, a heart was also found that had been conserved by adipocere.

Observations from more recent cases show that transformation of organs to adipocere is associated with long periods of immersion or burial. Burial of 6 years resulted in the conversion of the brain and heart along with subcutaneous tissue (Tanaka et al., 2016). As described previously a male submerged in a car for 7 years had the entire subcutaneous and internal organ converted into adipocere (Kasuda et al., 2016). In other cases of long submersion, there may be extensive soft tissue preservation but the organs are not converted into adipocere.

4.4. Bone

Even when a body has been skeletonised, adipocere may be found on the bone surfaces and within the bone marrow cavities (Delabarde, Keyser, Tracqui, Charabidze & Ludes, 2013; Henderson, King, Caffell & Allen, 2015). Pokines and Higgs (2015) found adipocere in 20% of cases where bones were recovered in or near the ocean. The adipocere was found adhered to the bone and embedded in exposed cancellous bone. Adipocere within bones is protected from surface erosion and can persist for some time.

Moses (2012) studied defleshed bovine bones buried in artificial soil which developed adipocere on the surface of the bone. This showed that a large amount of adipose tissue may not be necessary for formation to occur. Laboratory experiments on decomposition of bone found soft adipocere in bone marrow cavities submerged in pH 4 and pH 7 water while hard adipocere formed within the marrow cavities and surface of the bone in pH 10 water (Christensen & Myers, 2011).

Jopp-van Well et al. (2016) states that adipocere formation within bone is common in conventional burials. They present a case of an almost completely skeletonised corpse with extensive adipocere in the femur and humerus medullary cavities. The authors attempted to determine the PMI using radiocarbon dating in conjunction with knowledge on adipocere formation within bones. A possible period of death was determined as AD 1667 – AD 1952 through radiocarbon dating. The maximum PMI may be determined by macroscopic and microscopic identification of adipocere located throughout the bone but there is limited literature and the observations used are mostly accidental rather than from controlled experiments. In this case study of adipocere was not useful in further narrowing down the PMI estimated using radiocarbon dating. There is limited knowledge on bone adipocere formation and the PMI cannot currently be reliably estimated.

4.5. Summary

Formation throughout the body may be partial or complete. The time taken for adipocere to form is highly variable and depends on the environmental conditions as well as conditions within the body such as fat and water content. The most common area for adipocere to form is the subcutaneous fat of the body with formation in the cheeks, buttocks, abdomen and

breasts the most highly observed. Organ preservation depends on the state of putrefaction at the time of adipocere formation. The organs may be preserved by adipocere formation of the subcutaneous tissue or preserved as adipocere themselves. The brain is highly unlikely to form adipocere due to its observed rapid autolysis, however, two historical cases of brain adipocere have been confirmed. Formation was also confirmed in a heart. Bones may also develop adipocere on their surface or inside the medullary cavity. Estimation of the post-mortem interval using bone adipocere is limited by the current literature available.

5. Case studies involving adipocere

In this section, a table of case studies is presented detailing the year the case took place, the characteristics of the body (sex, weight, age), if the body was clothed, if insects were present, the PMI, location and distribution of adipocere, the environment the body was found in, cause of death and anything else notable about a particular case.

The case years that were reported ranged from 1950 to 2014. The PMI ranged from 21 hours up to an estimated 30-150 years. The age range was between 4 years and 78 years with the majority of the cases involving males. Most bodies exhibited subcutaneous formation of adipocere while only one showed complete adipocere transformation where both subcutaneous and internal organs where transformed into adipocere. Cases from water, soil and dry environments are all presented with the majority of cases found in water environments. Most bodies recovered from water were determined to have drowned, however in several cases death was due to gunshot injuries or sharp or blunt force trauma. Most bodies were clothed. Only six cases reported the presence of insects.

Table 5.1 Case studies involving adipocere formation

Case Year	1973	1973	__	1983	1983	2002	__	__	__	__	__
PMI	11 months	10 months	6 months	5 years	5 years	30-100 years	25 days	27 days	30 days	31 days	36 days
Sex/Age/ Weight	M, 20	F, 22	F	M	F	M, 30-45	M, 24	M, 27	M, 37	M, 23	F, 24
Adipocere location	Subcutaneous, cheeks, abdominal wall, thighs	Subcutaneous	Subcutaneous	Extensive subcutaneous, face, neck, trunk, extremities	Extensive subcutaneous, face, neck, trunk, extremities	Femur tubules	Limbs	Limbs and buttocks	Limbs and abdominal wall	Limbs and abdominal wall	Limbs, buttocks, abdominal wall
Environment	Water (fresh), cold, silt-prone sinkhole (South Australia)	Water (fresh), cold, silt-prone sinkhole (South Australia)	Soil (Sicily, Italy)	Water (lake, in vehicle), water temp.= >21˚ C at immersion (Minnesota)	Water (lake, in vehicle), water temp.= >21˚ C at immersion (Minnesota)	Water (lake), depth = 32 metres (Austria)	Water (sea), average water temperature = 11.07˚˚ C (Adriatic Sea)	Water (sea), average water temperature = 14.5˚ ˚C (Adriatic Sea)	Water (sea), average water temperature = 15.4˚˚ C (Adriatic Sea)	Water (sea), average water temperature = 11.7˚ ˚C (Adriatic Sea)	Water (sea), average water temperature = 14.5˚˚ C (Adriatic Sea)
Clothing (Y/N)	Y (full-length wetsuit)	Y (full-length wetsuit with buoyancy vest)	N	Unknown	Unknown	N	Y (fully)	Y (heavily)	Y (socks and shoes)	Y (wetsuit)	Y (underwear)
Presence of insects (Y/N)	N	N	N	N	N	N	N	N	N	N	N
Cause of death	Drowning	Drowning	Head trauma, blunt instrument	Unknown	Unknown	Unknown (skull fracture present)	Drowning	Drowning	Drowning	Drowning	Drowning
Notes	Internal organs putrefied	Internal organs putrefied	Dismembered, wrapped in black plastic bags, internal organs dehydrated	Skeletal muscles desiccated, organs well-preserved grossly but not histologically	Skeletal muscles desiccated, organs well-preserved grossly but not histologically	Present as skeletal remains only	Floating close to land	Floating in high seas	Floating close to land	Floating close to land	Floating close to land
Author (year)	Byard (2009)	Byard (2009)	Cascio and Teodoro (2014)	Cotton et al. (1987)	Cotton et al. (1987)	Darok et al. (2005)	De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)

—	—	—	2010	—	—	—	—	—	1995	1995	1995
58 days	64 days	118 days	6 months to 2 years	4 months	6 months	10 months	34 days	n.d	30 days	120 days	570 days
M, 38	M, 54	M, 42	M, YA	F, 50	M, 22	F, 50	M	F, 40-50	Unknown	Unknown	Unknown
Saponification	Saponification	Total saponification	Subcutaneous (pelvic girdle)	Slight formation	Slight to moderate formation	Marked formation over entire body	Subcutaneous, scalp	Extensive subcutaneous	Unknown	Unknown	Unknown
Water (sea), average water temperature = 10.8°° C (Adriatic Sea)	Water (sea), average water temperature = 15°° C (Adriatic Sea)	Water (sea), average water temperature = 11.8° °C (Adriatic Sea)	Bank of river (Strasbourg)	Water (lake) (Missouri)	Water (lake) (Missouri)	Water (lake) (Missouri)	Water (sea, tropholytic zone) (Mediterranean Sea)	Water (pond) (Delhi)	Water (fresh) (British Columbia)	Water (fresh) (British Columbia)	Water (fresh) (British Columbia)
Y (light)	Y (partial)	Y (heavily)	N	N	Y (fully)	Y (fully)	Y (flight suit)	N	Unknown	Unknown	Y
N	N	N	Y (*Muscina stabulan,* 2 empty pupae)	N	N	N	Y (larva)	N	N	N	N
Drowning	Drowning	Drowning	Drowning	Unknown	Gunshot wounds to head	Head trauma, blunt instrument	Helicopter crash	Cut throat	Unknown	Unknown	Unknown
Floating in high seas	Recovered on beach	Floating close to land	Incomplete skeleton, diazepam and nordiazepam detected	Weighted down with concrete blocks	Lesser degree of adipocere formation on areas covered by clothing	Lesser degree of adipocere formation on areas covered by clothing		Wrapped in plastic bag	Skin sloughing also present	Skin sloughing also present	
De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)	Delabarde et al. (2013)	Dix (1987)	Dix (1987)	Dix (1987)	Dumser and Türkay (2008)	Gupta and Jain (2011)	Hobischak and Anderson (1999)	Hobischak and Anderson (1999)	Hobischak and Anderson (1999)

1996	1996	1994	—	—	—	2011	1998	1950	2008	2010	2007
91 days	180 days	3 months	13 months	Unknown (assessment of adipocere: 30-150 years)	7 years	3 years	4 years	6 years	8 months	5-7 months	65-66 hours
Unknown	Unknown	M, 24, 67kg	M, 78	M, 30-50	M, 50s, 52.3kg	M, 35	M, 55	M, 40s	M	M	M, 35
Unknown	Unknown	Subcutaneous, skeletal muscles	Complete subcutaneous	Medullary cavities of femur and humerus, compact and cancellous bone	Complete subcutaneous, muscles and internal organs	Right palm	Complete subcutaneous	Complete subcutaneous	Brain, bone marrow, left foot	Pelvis and lower limbs	Cheeks,front/back of trunk, upper limbs except hands, lower limbs except feet
Water (fresh) (British Columbia)	Water (fresh) (British Columbia)	Water (river, within vehicle)	Water (river)	Tideland of river (River Elbe, Otterndorf)	Water (lake), depth = 40 metres, water temp. = 25˚˚ C at time of immersion	Soil (loamy), depth = 1.8 metres (Warsaw, Poland)	Soil, depth = 4 metres	Water (fresh) (Lake Orta, Italy)	Water (well, ground water) (Piedmont, Italy)	Water (well, ground water) (Sicily, Italy)	Water (marshy pond)
Y	Unknown	Unknown	Unknown	N	Unknown	N	Unknown	Y	Y (clothing and shoes)	Y (trousers)	Y
N	Y (*Hydrotaea Robineau-Desvoidy,Piophilidae,Creophilus,*Necrodes)	N	N	N	N	N	N	N	Y (Calliphoridae, Fanniidae, Muscidae, Trichoceridae, Sphaerocerdidae, Psychodidae, Nematocera)	Y (Syrphidae, Muscidae, Cleridae)	N
Unknown	Unknown	Drowning	Drowning	Unknown	Unknown	Strangulation	Gunshots to head	Gunshots to head	Drowning	Unknown	Gunshot injuries
	Skull and lower mandible on sand, rest of body found in pool of water	Toluene was detected within adipocere	Adipocere visualised using multislice computed tomography	Skull and partial thorax	Complete adipocere formation	Left hand uncovered, right hand gloved, right palm and finger ridges preserved		Skin and subcutaneous tissue rapidly falling off body after retrieval		Entomological evidence collected 20 months after body recovery	
Hobischak and Anderson (1999)	Hobischak and Anderson (1999)	Inoue et al. (1996)	Jackowski et al. (2005)	Jopp-van Well et al. (2016)	Kasuda et al. (2016)	Klemczak et al. (2015)	Kobayashi et al. (1999)	Lentino (1956)	Magni et al. 2013	Magni et al. 2013	Mohan Kumar et al. (2009)

—	—	—	—	2013	2013	2013	2013	2013	2013	2013	2013
4 months	5 months	4 years	7 months	1 day 20 hours	2 days 7 hours	1 day 15 hours	3 days 5 hours	1 day 17 hours	1 day 4 hours	3 days 5 hours	2 days 3 hours
M, 41	M, 42, 45kg	F, 77	M, 65, 50.4kg	M, 42	M, 35	F, 59	F, 5	M, 47	M, 55	M, 37	M, 4
Subcutaneous, upper body	Marked formation, brain	Almost complete subcutaneous and visceral	Subcutaneous, head, neck, right shoulder, upper torso, left leg	Front and back of trunk, upper limbs except hands	Front of trunk, upper limbs except hands	Face, front of trunk, upper limbs, lower limbs	Face, front and back of trunk, upper limbs, lower limbs	Abdomen, upper limbs, lower limbs except feet	Front and back of trunk, upper limbs except hands, lower limbs except feet	Front of trunk, sides of back, arms	Front of trunk, upper limbs except hands
Soil (graveyard), depth = 85 centimetres	Water (sea)	Dry, clothes box covered with plastic bags	Soil (sandy), depth = 30-42 centimetres, pH 2.6-4 (Belgium)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)
Y (short-sleeved shirt, trousers)	Y	Unknown	N	Y	Y	Y	Y	Y	Y	Y	Y
N	N	N	Y (*Calliphora vicina,* larvae and pupae)	N	N	N	N	N	N	N	N
Gunshot to abdomen	Drowning	Unknown	Smothering	Hanging	Unknown	Unknown	Drowning	Alcohol intoxication	Drowning	Septicaemia from burn	Unknown
Buried above an old grave, head buried under cement, lower body skeletonised	Nicotine and cotinine detected	Detected novel fatty acid of adipocere: *cis*-12-octadecenoic acid	Differential decomposition (desiccation, adipocere and decomposition)								
Mohd Nor and Das (2012)	Nishimura et al. (2009)	Nushida et al. (2008)	Schotsmans et al. (2011)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)

2013	2013	2013	2014	2014	2014	2014	2014	—	—	—
2 days 9 hours	1 week	3 days 3 hours	6 days	3 days	21 hours	3 days	6 days	>6 months	>6 months	5-6 years
M, 38	M, 14	M, 33	F, 24	M, 35	M, 13	F, 29	M, 45	F, 20-30	M. >60	M, 20s
Abdomen, chest, arms	Face, front/back of trunk, upper limbs except hands, lower limbs except feet	Back of trunk, right upper limb except hand, front of right thigh	Face, front and back of trunk, upper limbs, lower limbs	Front of trunk, upper limbs except hands, front of lower limbs except feet	Abdomen, lower back, left upper limb except hand	Face, left side of trunk, lower back, arms, lower limbs	Front of trunk, left side of back, upper right thigh	Base of skull	Right side of skull, scalp, fractured ends of hyoid	Subcutaneous except head and extremities, brain, heart, muscles
Water (river bank, hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (closed box inside shop, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Soil	Soil	Soil, depth = 1 metre
Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y
N	N	N	N	N	N	N	N	N	N	N
Drowning	Drowning	Head injury	Multiple stab wounds	Drowning	Strangulation	Cut throat	Unknown	Strangulation	Strangulation	Cardiac injury
								Only skull present with fragments of hyoid bone embedded in adipocere	Skull, long bone fragments and decomposed tissue present	Toluene detected at high levels, unclear what effects this had on death
Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	S. Smith (1939)	S. Smith (1942)	Tanaka et al. (2016)

n.d = not determined; F = female; M = male; YA = young adult

6. Discussion and Conclusions

Knowledge pertaining to adipocere has been gathered for several hundred years and, as stated by Ubelaker and Zarenko (2011), there has been much insight into the various aspects of adipocere formation.

Adipocere formation and the timing of that formation is highly variable throughout the body and is influenced by a wide variety of factors both environmental and body specific. Development of reference material outlining the timing and distribution of adipocere formation throughout the body may help forensic professionals in casework. This would include the different environments in which adipocere may be formed and the variables within them that may have influenced adipocere formation e.g. temperature and pH level.

After the latest review by Ubelaker and Zarenko (2011) research has continued into the factors that influence the formation of adipocere along with those that may promote its degradation.

The key to adipocere's persistence has been cited as the absence of oxygen. In the presence of oxygen, adipocere will degrade and decomposition of the remains will continue (Schoenen & Schoenen, 2013). An additional pathway of adipocere degradation under poikiloaerobic conditions has been proposed (Fiedler et al., 2015). Research surrounding the degradation of adipocere is still very limited.

The estimation of post-mortem interval (PMI) is still complicated by the presence of adipocere and no reliable method has yet been developed to address the problem. Skin slippage was observed to enhance the formation of adipocere in rabbits (Widya et al., 2012) and as skin slippage is used in PMI estimates this is a potential factor that, with further research, may improve PMI estimates.

Recent case studies of interest include the observation of whole body adipocere formation, which is extremely rare, and the discovery of the preservation of a large number of human brains through adipocere formation (Kasuda et al., 2016; Serrulla et al., 2016).

Study of the elemental composition of adipocere may aid in further distinguishing adipocere samples into the different stages of formation, early, intermediate and late stage, and may also be useful to differentiate between water environments in which adipocere has formed. Chemical analysis of the environment in which adipocere forms is also necessary to further understand the variables, such as pH, bacteria and water flow, that influence formation (Stuart et al., 2016).

Further research in the area of adipocere degradation is suggested to focus on the biochemical pathways and environmental influences that govern adipocere formation (Schoenen & Schoenen, 2013).

Further study of bacteria associated with formation and degradation of adipocere is needed as research around this area is also limited. DNA analysis of bacteria found in association with adipocere could be utilised to determine species and their relative abundance (Ueland et al., 2014).

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Part Two – Manuscript

Review of Adipocere Formation on Decomposing Bodies

Abstract

Adipocere is a substance formed from the decomposition of adipose tissue and represents a disruption to the typical decomposition process. This disruption causes decomposition to slow or arrest completely, placing a body into a state of preservation. Adipocere formation causes complications in the estimation of the time since death (Post-Mortem Interval, PMI) and there is still no reliable model to assess the PMI of a body exhibiting adipocere formation. The study of adipocere involving both laboratory-based and case study-based research is important to improve PMI estimations and to aid pathologists and investigators during a case.

This review presents an update on the knowledge surrounding the chemistry of adipocere formation, the factors that affect adipocere formation, the timing and distribution of adipocere formation, and presents a table of case studies involving adipocere formation that could be used as a reference for forensic professionals in cases.

Keywords: adipocere, case studies, decomposition, post-mortem interval (PMI)

1. Introduction

Following death, a body will start the natural process of decomposition. Generally, the decomposition process is expected to constitute several subsequent stages that will modify the body from fresh to skeletonised remains (Payne, 1965). The rate of decomposition is affected by both the external environment and the body itself (e.g. size, amount of fat tissue, cause of death) (Pinheiro, 2006). The stage of decomposition can be used along with other indicators, such as the presence of certain insects, to estimate the time since death or Post-Mortem Interval (PMI). The PMI is an estimation of the time between death to the time the body is discovered. Estimation of the PMI is important in a forensic investigation as it may aid in the identification of a body, be used in the reconstruction of the events prior to death, and it may corroborate or contradict the alibi of a suspect (Madea, 2016; Thali et al., 2011). PMI estimation is more difficult when the typical decomposition process is disrupted, such as in the case of the formation of adipocere (Vass, 2011).

The term 'adipocere' was first introduced by Fourcroy in 1789, although Browne, in 1656, may have been the first to have made a reference to it (Barnes, 1934). Adipocere is a substance that forms on decomposing remains through the decomposition of adipose tissue found within a body. It represents an alteration of the decomposition process, slowing it down or halting it up to hundreds or even thousands of years under stable conditions (Aufderheide, 2011).

Adipocere hinders grave reuse in countries where cemeteries carry out this practice. Graves are generally left for 15-25 years during which the body should undergo complete decomposition (Fiedler & Graw, 2003). This process is hindered by adipocere and a grave may be opened with the discovery of a partially or fully preserved body still inside. In a forensic

context, the study of adipocere is important because of this property of inhibiting decomposition (Forbes, Stuart, Dadour & Dent, 2004). Adipocere causes issues with determining the PMI but it may aid investigations by allowing identification of an individual due to preserved facial or body features, aid in the determination of the cause and manner of death due to preserved injuries, inform investigators about the type of environment it has formed in, and aid investigators in predicting what condition to expect a body to be in which may aid in better recovery of remains (Christensen & Myers, 2011; Mohan Kumar, Monteiro, Bhagavath & Bakkannavar, 2009; Pinheiro, 2006; Ubelaker & Zarenko, 2011).

The process of the formation of adipocere, the type of adipocere formed, and the distribution of adipocere within a body varies case by case and environment by environment, making every case unique. For this reason, the study of previous cases that involve adipocere may aid forensic investigations. If the features of a case and case study are similar the processes and techniques used in the case study could be used as a reference for the current case. Research into adipocere formation is pivotal as it is important to be aware of the various environments adipocere forms in, the factors that influence adipocere formation, and the timing of its formation as this may assist in the determination of the PMI.

Review of the literature on adipocere formation will provide an update on the present knowledge of the chemical composition of adipocere, the mechanism of its formation, the numerous factors affecting its formation, and its formation throughout the body. The construction of a table detailing case studies involving adipocere formation will also provide forensic professionals with a reference to cases that could be referred to in relation to a current case.

2. Chemistry of adipocere formation

The most extensive research regarding adipocere has been on its chemical composition and investigations into the mechanism of its formation.

Due to restrictions on the use of human remains in research, pigs (*Sus scrofa* L.) have been used as the next best model of human decomposition. A study conducted by Notter and collaborators (2009) found that pigs were suitable to be used as models in the investigation of adipocere but considerations needed to be taken regarding differences between the composition of adipose tissue and the process of adipocere formation in pigs compared to humans. These differences cause a margin of error which affects the application of the pig study findings to human adipocere formation.

2.1. Analytical techniques for adipocere investigation

In order to study adipocere formation there need to be reliable and relatively simple techniques available. A common technique used to study adipocere is gas-chromatography-mass spectrometry (GC-MS) (Adachi et al., 1997; Bereuter, Lorbeer, Reiter, Seidler & Unterdorfer, 1996; Bereuter, Reiter, Seidler & Platzer, 1996; Forbes, Stuart & Dent, 2002; Forbes, Keegan, Stuart & Dent, 2003). Other techniques that have been utilised for adipocere research include: attenuated total reflectance infrared spectroscopy (ATR-IR) (Bereuter, Mikenda & Reiter, 1997; Stuart, Craft, Forbes & Dent, 2005), diffuse reflectance infrared spectroscopy (Stuart et al., 2000), Fourier transform infrared spectroscopy (FTIR) (Forbes et al., 2004), solid phase extraction (Notter, Stuart, Dent & Keegan, 2008), liquid chromatography-mass spectrometry (LC-MS) (Algarra, Rodriguez-Borges & Esteves da Silva,

2010), inductively coupled plasma-mass spectrometry (ICP-MS) (Forbes, Stuart & Dent, 2005a, 2005b; Stuart, Notter, Dent, Selvalatchmanan & Fu, 2016) and lyophilization (Liu, Park, Monsalve & Chen, 2010).

ATR-IR has the advantage of being a less destructive technique compared to GC-MS and absorption bands relating to triglyceride and C=O stretching that are visualised using this technique may be utilised to determine the stage of adipocere formation. However, when analysed with ATR-IR, adipocere samples must either be extracted and cast as a film to be analysed or made up into a solution for analysis (Bereuter et al., 1997; Stuart et al., 2000; Stuart et al., 2005). Diffuse reflectance infrared spectroscopy allows the detection of structural isomers and the use of powdered or soil samples containing adipocere and has easy sample preparation (Stuart et al., 2000). FTIR is able to detect triglycerides and salts of fatty acids within adipocere in contrast to GC-MS (Forbes et al., 2004). ICP-MS was used to identify the type of cations and their percentage in the fatty acid salts of adipocere (Forbes et al., 2005a; 2005b; Stuart et al., 2016). LC-MS has been used to detect free fatty acids in soil (Algarra et al., 2010). Solid phase extraction has also been used alongside GC-MS as a quantitative technique for identifying free fatty acids (Notter et al., 2008). Another technique used in conjunction with GC-MS was lyophilization which increases the yield of fatty acids recovered from a sample. Tissue samples are converted into a fine powder to which hexane is added and the resulting sample is analysed with GC-MS (Liu et al., 2010).

Adipocere has also been visualised using multislice computed tomography which produced an image of the distribution of adipocere throughout a body whose subcutaneous tissue was almost completely transformed into adipocere (Jackowski et al., 2005).

The technique used to analyse an adipocere sample will depend on the type of sample (e.g. soil), type of result required (e.g. quantitative or qualitative, specific component of

adipocere to be analysed/identified). A comparison of the techniques used for adipocere analysis is shown in Table 2.1.

Table 2.1 Comparison of techniques that have been used to analyse adipocere

Technique	Type of sample	Sample preparation	Type of result	Author (year)
Gas chromatography-mass spectrometry (GC-MS)	Tissue, Soil	Extraction and conversion into methyl esters of fatty acids; Extraction and conversion into trimethylsilyl esters of fatty acids; Lyophilization, extraction and conversion into trimethylsilyl esters; Solid phase extraction	Quantitative and qualitative detection of fatty acids	Adachi et al. (1997); Bereuter and Lorbeer et al. (1996); Bereuter and Reiter et al. (1996); Forbes et al. (2002); Forbes et al. (2003); Forbes et al. (2004); Liu et al. (2010)
Liquid chromatography-mass spectrometry (LC-MS)	Soil	Extraction and conversion into amide derivatives of fatty acids	Qualitative detection of fatty acids	Algarra et al. (2010)
Attenuated total reflectance infrared spectroscopy (ATR-IR)	Tissue, Soil	Cast into film or made up into solution	Semiquantitative detection of triglycerides, fatty acids and fatty acid salts	Bereuter et al. (1997); Stuart et al. (2005)
Diffuse reflectance infrared spectroscopy	Soil	Sample ground with powdered KBr	Qualitative detection of fatty acids, triglycerides and fatty acid salts	Stuart et al. (2000)
Fourier transform infrared spectroscopy (FTIR)	Tissue, Soil	Sample ground with powdered KBr	Qualitative detection of triglycerides, fatty acids and fatty acid salts	Forbes et al. (2004)
Multislice computed tomography	Whole body	None	Image of adipocere distribution throughout a body	Jackowski et al. (2005)
Inductively coupled plasma-mass spectrometry (ICP-MS)	Tissue	HNO_3, HCl and H_2O_2 added, diluted with deionised water	Type and abundance of cations of fatty acid salts	Forbes et al. (2005a, b); Stuart et al. (2016)

2.2. Chemical composition of adipocere

Adipocere consists mainly of saturated fatty acids including palmitic, myristic and stearic acid. Besides these, hydroxy fatty acids (10-hydroxystearic and 10-hydroxypalmitic), fatty acid salts of magnesium, calcium, sodium and potassium, unsaturated fatty acids including palmitoleic, oleic and linoleic acid, along with triglycerides and oxo fatty acids (10-oxostearic acid and 10-oxopalmitic acid) have also been found within adipocere (Adachi et al., 1997, Forbes et al., 2004, Stuart et al., 2000, Takatori & Yamaoka, 1977b). Minor components that have been found in some samples of adipocere include 10-hydroxy-12-octadecenoic acid, *cis*-12-octadecenoic acid, epicoprostanol, 9-chloro-10-methoxy palmitic acid (9-methoxy-10-chloro palmitic acid), and 9-chloro-10-methoxy stearic acid (9-methoxy-10-chloro stearic acid) (Adachi et al., 1997; Nushida, Adachi, Takeuchi, Asano & Ueno, 2008; Takatori, 2001; Takatori, Terazawa, Nakano & Matsumiya, 1983).

Analysis of volatile organic compounds (VOCs) associated with adipocere found that adipocere contains 18 VOCs (Hoffman, Curran, Dulgerian, Stockham & Eckenrode, 2009).

Research into the chemical composition of adipocere has been important as there have been several components of adipocere that have been used as indicators of adipocere formation (Bereuter & Lorbeer et al., 1996; Bereuter & Reiter et al., 1996; Yan, McNally, Kontanis & Sadik, 2001). These include oleic acid, hydroxystearic acid, and palmitic acid. These indicators and their relative percentage in adipocere are used in research and case studies to positively identify the presence of adipocere (Bereuter, Lorbeer et al., 1996; Forbes et al., 2005b; Serrulla et al., 2016). Classification of adipocere into various stages of formation has also been achieved using the chemical composition of adipocere (Forbes et al., 2004).

2.3. Mechanism of adipocere formation

The current knowledge on the mechanism of adipocere formation is still limited and there is no single accepted model of formation.

Here we divide the mechanism of formation into chemical-based and microbiological-based. However, adipocere is the result of a combination of these two processes.

a. Chemical mechanism for adipocere formation

It is known that adipocere formation is the result of the post-mortem decomposition of adipose tissue and is facilitated by intrinsic lipases as well as the enzymatic activity of micro-organisms that originate from within the intestines and respiratory system. Aerobic organisms initially utilise the oxygen present in the tissues which causes an anaerobic environment to form and favours the survival of anaerobic organisms that are associated with adipocere formation (Evans, 1963).

Adipose tissue contains lipids, of which 90-99% are triglycerides. Triglycerides are composed of a glycerol molecule with three fatty acid molecules attached (Dent, Forbes & Stuart, 2004). Decomposition of adipose tissue causes the release of triglycerides which are then converted into other components of adipocere. The fatty acids contained within adipose tissue are mainly unsaturated fatty acids that make up 65% of the total content. Overall, oleic acid (~45%) is the most abundant unsaturated fatty acid followed by linoleic acid (~15%). Palmitic acid (~20%) is the most abundant saturated fatty acid (Hodson, Skeaff & Fielding, 2008). In contrast, the main components of adipocere are saturated fatty acids and palmitic acid is the most abundant (Bereuter & Lorbeer et al., 1996). The overall process of adipocere formation is shown in Figure 2.1. The process involves the hydrolysis of triglycerides into

saturated and unsaturated fatty acids and the hydrogenation of the unsaturated fatty acids into saturated fatty acids (Forbes et al., 2004). Lipases within cells initiate the hydrolysis of the triglycerides for a brief period after which bacterial enzymes facilitate more extensive hydrolysis. If there is a sufficient concentration of enzymes and water the process will continue until most or all triglycerides are depleted. Water may be derived from the environment or from the body itself, either within the fatty tissue or drawn from surrounding tissue by diffusion (Evans, 1963).

In addition to unsaturated and saturated fatty acids, adipocere also contains fatty acid soaps, hydroxy fatty acids and oxo fatty acids that are produced as by-products (Forbes et al., 2004).

Sodium ions in the interstitial fluid and potassium in cell water may interact with the fatty acids released from adipose tissue to form their respective fatty acid soaps (Forbes et al., 2004). According to Vass (2001), adipocere will be hard and crumbly if sodium soaps are formed, and soft with a paste-like consistency with potassium soap formation. The environment a body is placed in will also contain metal ions, such as calcium and magnesium, that can displace the sodium or potassium ions (Dent et al., 2004). Powers (2005) states the opposite characteristics, the soap being hard and crumbly if potassium soaps are formed and soft and paste-like if sodium salts are formed, but that if sodium is displaced by calcium it will produce a more brittle substance. Ruttan and Marshall (1917) noted that hydroxystearic acid was characteristic of adipocere and may be derived from oleic acid through hydration. Hydroxy acids are only detected in adipocere samples and not adipose tissue samples (Takatori & Yamaoka, 1977a).

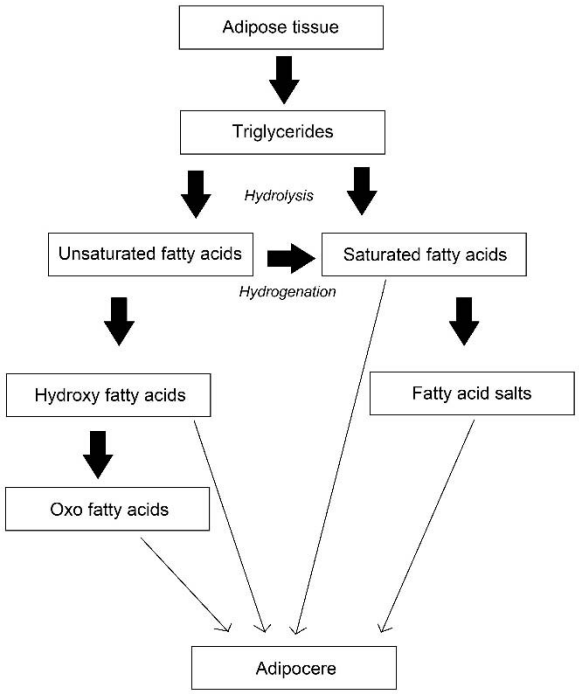

Figure 2.1 Flow diagram of the process of adipocere formation

It has been suggested that fatty acids with high melting points e.g. hydroxy fatty acids, oxo fatty acids, stearic acid and palmitic acid that are contained within adipocere are important to the formation and stability of adipocere (Serrulla et al., 2016; Takatori, 1996; Takatori & Yamaoka, 1977b). Schoenen and Schoenen (2013), however, oppose this view and state that studies have not taken microbial activity into account. The amount of oxygen necessary for complete decomposition by respiration is not available in environments conducive to adipocere and incomplete decomposition through fermentation occurs. Glycerol cleaved from triglycerides is degraded to carbon dioxide and water in respiration, while fermentation results in soluble alcoholic components that may wash away in the environment. Respiration degrades fatty acids through beta-oxidation into carbon dioxide and water but fermentation of fatty acids cannot occur and fatty acids are converted to polyhydroxy acids, a form of energy storage. These acids are insoluble in water and along with fat and long-chain fatty

acids are not washed away. They remain where they originate in the body. The authors state that lack of oxygen is the true cause of adipocere formation and preservation.

Three main theories have previously been suggested to explain the process of adipocere formation. These theories explain some observations of adipocere formation but not all aspects of it (Bereuter & Lorbeer et al., 1996; Takatori & Yamaoka, 1977a):

- The *fat migration theory* states that fat spreads throughout the surrounding tissue as decomposition of adipose tissue occurs. Triglycerides contained within the fat are broken down and the resulting free fatty acids, e.g. oleic acid, and glycerol diffuse or separate out due to gravity.
- The *saponification theory* states that the main reaction of formation is the hydrolysis of fat into fatty acids and glycerol. Insoluble soaps of the fatty acids are then formed. These soaps only constitute 2-35% of the total adipocere composition and saponification is not thought to play a major role in adipocere formation (Takatori & Yamaoka, 1977a).
- The *hydrogenation theory* states that fatty acids are released and spread into adjacent tissues. Unsaturated fatty acids are the main component and are hydrogenated into saturated fatty acids. This theory is more widely supported through research but results from several studies have shown contradictions. The theory requires a reductive state in order for adipocere to form but research on an adipocere tissue sample from Ötzi, the Tyrolean Iceman carried out by Bereuter and Reiter et al. (1996) found that the tissue sample was in a high oxidational state and Adachi et al. (1997) found the presence of epicoprostanol in their adipocere sample which indicated both oxidation and reduction had occurred.

Yan et al. (2001) produced a preliminary model for adipocere formation using pigs immersed in several water environments. Oleic acid was detected hours after each pig was immersed in water and the formation of adipocere followed the general trend of a decrease in oleic acid with a related increase in the level of hydroxystearic acid as time since immersion increased. The authors suggested that the ratio of oleic to hydroxystearic may be able to be used in improving time of death estimates, but that validation studies with human samples would need to be carried out as well as looking at factors that may influence the ratio. A theory as to the formation of hydroxystearic acid from oleic acid was also presented. Oleic acid was observed to decrease as hydroxystearic acid increased and this suggested that there was conversion of oleic acid into other derivatives, though the method of conversion is not clear. They suggested that adipocere formation may be due to partial hydrogenation where some double bonds are saturated and some undergo stereomutation or bond migration. In the case of oleic acid, hydrogenation would produce stearic acid, stereomutation would produce [R-(Z)-12-hydroxycis-9-octadecanoic while hydration produces hydroxystearic acid and dehydrogenation of hydroxystearic results in oxostearic acid.

b. Microbiological mechanism for adipocere formation

Many microorganisms including *Bacillus subtilis*, *Micrococcus luteus, Staphylococcus aureus*, some *Pseudomonas* species and *Clostridium perfringens* can convert oleic to hydroxy fatty acids. *Micrococcus luteus*, *Flavobacterium meningosepticum* and other *Pseudomonas* species can also convert hydroxy fatty acids to oxo fatty acids. This suggests that the bacteria have both cis-9-enoic acid hydrase and 10-hydroxy fatty acid dehydrogenase (Gotouda, Takatori, Terazawa, Nagao & Tarao, 1988; Takatori, Gotouda, Terazawa, Mizukami & Nagao, 1987). Fatty acids with a cis-9-unsaturation, as is the case with myristoleic, palmitoleic and oleic fatty

acids, can be converted into both hydroxy and oxo fatty acids. Linoleic can only be converted to hydroxy fatty acids. Oxo fatty acids are not converted to hydroxy fatty acids by any of the bacteria and therefore oxo fatty acids are derived from hydroxy fatty acids which are in turn derived from oleic acid.

c. Stages of adipocere formation

Another adipocere formation model that has been proposed uses the percentage of the different components of adipocere to classify formation into early, intermediate and advanced stages (Forbes et al., 2004).

Early stage formation indicates only minor tissue decomposition and the composition is close in similarity to adipose tissue with triglycerides still present but with a decreased unsaturated fatty acid content and increased saturated fatty acid content.

Intermediate stage formation shows a further increase in palmitic acid and decrease in oleic acid with the addition of increased stearic acid content. Triglycerides are still detectable at this stage.

Advanced stage adipocere is indicated by palmitic acid comprising more than 50% of the total adipocere composition. Oleic acid is even more reduced and other unsaturated acids and triglycerides are no longer present.

These stages are not distinct and one sample may fit into more than one stage, depending on which component is assessed. Classification into each stage is also not dependent on the length of decomposition and other factors must enhance or inhibit formation. Stuart et al.

(2000) found similar results with a 26-month-old forensic sample having a comparable composition to a 27-year-old sample.

Notter and Stuart (2012) used the total percentage of saturated fatty acids to monitor the different stages. Early stage was 40-60%, intermediate was 70-90% and advanced was more than 90%.

Further discussion of adipocere formation in regard to the timing and distribution of adipocere formation within the body as well as the factors affecting adipocere formation and case studies involving adipocere formation are contained in sections 3, 4 and 5.

2.4. Degradation of adipocere

2.4.1. Chemical and microbiological degradation

Adipocere is able to withstand hundreds and even thousands of years in various environments but if exposed to certain conditions adipocere will degrade. Anaerobic conditions are conducive to adipocere formation and aerobic conditions promote the degradation of adipocere. Adipocere degradation has not been well studied and more research is needed in this area.

The half-life of adipocere is calculated to be 11-82 years under anaerobic conditions, 0.7-10 years under aerobic conditions and 1.2-2.1 years with burial in active soils (Fründ and Schoenen, 2009). Both Gram-negative bacteria – *Pseudomonas*, *Serratia*, *Alcaligenes*, *Enterobacter* and Gram-positive bacteria – *Bacillus*, *Nocardia*, *Cellulomonas* have been associated with adipocere. Gram-positive bacteria hydrolyse the adipocere and resistance of

adipocere to degradation is due to Gram-positive bacteria exclusion from the formation environment (Pfeiffer, Milne & Stevenson, 1998).

Schoenen and Schoenen (2013) noted that degradation can only occur on the surface of the adipocere as there is a lack of oxygen within it. They cited another study in which the weight loss of adipocere samples was observed to be between 7.6% and 45.3% after a year. Adipocere degradation will be a prolonged process due to the insufficient exchange of oxygen in the environment.

Fiedler, Berns, Schwark, Woelk and Graw (2015) examined suspected degraded adipocere in which adipocere was observed to contain higher carbon and hydrogen levels while the degraded material had higher sulphur, nitrogen and oxygen. The authors postulated that in addition to aerobic degradation, poikiloaerobic conditions can degrade adipocere within burial environments. Poikiloaerobic conditions are conditions in which there is an alternating aerobic and anaerobic environment. Autoxidation of fatty acids, use of alternative electron acceptors to produce iron sulphide, and the Maillard reaction that produces melanoidins were three reactions suggested to occur under these conditions.

2.4.2. Degradation of adipocere mediated by macrofauna

In addition to chemical and microbial degradation, adipocere can also be degraded by macrofauna. Macrofauna found to be associated with adipocere degradation are the fly genus, *Piophila* (Diptera: Piophilidae) and beetle species, *Omosita colon* (L.) (Coleoptera: Nitulidae).

Piophila are mostly scavengers that are found on decomposing bodies around 3-6 months into the decomposition process. They are found when fatty acids are present or may be

found later when well-marked adipocere formation is present (Smith K. G. V., 1986). In a study looking at insect succession on pigs, *Piophila* species were found feeding on the adipocere and were observed to cause the adipocere to become foamy. They appeared from 90-92 days after death to 336 days after death and were observed on pigs that were left in the sun and pigs placed in the shade (Anderson, Hobischak, Beattie, & Samborski, 2002).

In China, a study conducted on beetle succession of pigs observed the species *O. colon*, a saprophagous beetle, in association with adipocere. *O. colon* is widely distributed throughout China and found in bodies in an advanced stage of decomposition. It was observed to break down the adipocere slowly and the authors suggested that it may represent an important forensic indicator, especially in relation to adipocerous bodies (Lyu, Wan, Yang, Tang, & Xu, 2016).

3. Factors affecting adipocere formation

Adipocere formation is dependent on many factors within the external environment and within the internal environment of the body. Water, soil and dry environments may all be conducive to adipocere formation depending on other factors within these environments. These factors influence formation by enhancing or inhibiting adipocere formation and may also influence its composition. These factors will also influence the time of death estimates and at least some need to be considered when adipocere formation is present. Adipocere formation in environments has been described as typical or atypical (Nushida et al., 2008). Typical conditions were identified as water immersion, wet graves and damp vaults while atypical conditions were dry conditions. Adipocere forms more than 10 times slower in atypical conditions. The most agreed upon factors that are most conducive to adipocere formation in any environment are sufficient adipose tissue, mildly alkaline pH, moisture, anaerobic conditions, warm temperatures and the presence of bacteria (O'Brien & Kuehner, 2007; Ubelaker & Zarenko, 2011).

There are a huge number of different factors that may affect the formation of adipocere on a body and taking each and every one into consideration is difficult, as they never appear in isolation. However, conducting research to observe factors that affect the formation of adipocere is useful in giving investigators as much information as possible to aid them in an investigation into a death where adipocere is present.

3.1. Aquatic environments

Formation of adipocere may occur in a range of different water sources including seawater, rivers and lakes (Adachi et al., 1997; Heaton, Lagden, Moffatt & Simmons, 2010), bathtubs and wells, in warm, tropical waters and in cold seawater or melting glaciers (Bereuter & Lorbeer et al., 1996; Forbes, Wilson & Stuart, 2011; O'Brien & Kuehner, 2007). The factors that have been proposed to enhance and inhibit adipocere formation in water are listed in Table 3.1.

Table 3.1 Factors that inhibit and enhance adipocere formation in water environments.

Factor	Enhance	Inhibit	Author (year)
Temperature	21˚C to 45˚C	Less than 21˚C	Forbes et al. (2011)
Clothing/ material	Natural fibre material (wool, cotton) Synthetic fibre material (polyester, acrylic)	Absent	Notter and Stuart (2012); O'Brien and Kuehner (2007)
Skin slippage	Present	Absent	Widya, Moffatt and Simmons (2016)
Scavengers/ insects	Absent	Present	Forbes et al. (2011); Ueland, Breton and Forbes (2014)
Water type	River Distilled	Seawater Chlorine Saline Deionised	Stuart et al. (2016); Yan et al. (2001)
Submersion	Complete submersion	Partial submersion	Notter et al. (2009); Ueland, Breton and Forbes (2014)
Bacteria	Gram-negative Gram-positive (early stages)	Lack of Gram-positive bacteria at early stages	O'Brien and Kuehner (2007); Ueland, Breton and Forbes (2014)

3.1.1. Temperature

The main influence on decomposition in an aquatic environment is the water temperature (Heaton et al., 2010). Adipocere forms in a variety of different temperatures but at a variable rate. Rates of 12-18 months in 4˚C and 2-3 months in waters of 15˚C to 22˚C have been reported along with faster rates of 38 days in 10˚C to 12˚C seawater and 3 months in 13˚C water (Forbes et al., 2011; O'Brien & Kuehner, 2007). Formation of adipocere is enhanced by warm temperatures between 21˚C and 45˚C but adipocere may also form in cold water at a reduced rate. Cool temperatures between 7˚C and 16˚C promote adipocere formation into early or intermediate stages but if exposed to colder temperatures, it will not be allowed to develop into an advanced stage (Forbes et al., 2011).

Warm temperatures are thought to enhance formation because the activity of *Clostridium perfringens*, a major bacteria associated with adipocere formation, is at its optimal between these values. Bacterial enzymes released by *C.* perfringens remain present in the water after temperatures drop below the optimum range, ceasing bacterial growth, and this is thought to be the reason that adipocere formation still occurs at sub-optimal temperatures (Cotton, Aufderheide & Goldschmidt, 1987).

3.1.2. Submersion

Complete submersion in an aquatic environment is conducive to formation as it is likely to prevent contact with insects (but not to other organisms, see Anderson, 2010) and delay decomposition due to cooler temperatures underwater. Forbes et al. (2011) found that the depth of submersion did not influence formation. Insects and scavengers are more likely to have access to a floating body and the body is more likely to be swept along by currents, both

of which inhibits adipocere formation (Dirkmaat, 2012; Notter et al., 2009; Ueland et al., 2014).

In an experiment aiming to simulate field conditions, O'Brien and Kuehner (2007) placed three human bodies (referred to as Body 1,2 and 3) in water-filled pits containing water from an underground pipeline. This experiment was performed in Knoxville, Tennessee at the Anthropological Research Facility (ARF). Liquid and tissue samples from each body were collected using a syringe with a needle and were analysed for fatty acids and microbes. Body 1 had perimortem open trauma, algal growth, and was submerged the entire experiment. Adipocere did not form on this body and it was determined that there was no C. *perfringens* on the body. Body 2 and 3 remained floating the entire experiment and adipocere formed on both bodies after 3 months. Signs of adipocere formation appeared around 6 weeks into the experiment within the water-level zone. Early formation of adipocere was noted at 6-8 weeks, increased formation at 8-10 weeks, and advanced formation at 10-12 weeks. Complete submergence, while favourable, was shown not to be necessary for adipocere formation, as the two bodies that did exhibit adipocere formation remained floating the entire experiment. The absence of *C. perfringens* was also shown to inhibit formation. The final observation was that although the temperature varied throughout the experiment adipocere was still able to form and therefore stable temperatures were not necessary for adipocere formation. The limit of this experiment was the fact that the initial conditions of the three bodies were not the same, and the small sample size was not sufficient for a statistical analysis.

3.1.3. Type of water

Yan et al. (2001) studied distilled, chlorinated and saline water to observe oleic acid degradation and hydroxystearic formation in the formation of adipocere. Distilled water showed the highest degradation rate while saline was the slowest. Conversion to hydroxystearic was most rapid in distilled water and the least in chlorine. Saturation of oleic degradation, the point where the rate of degradation did not increase further, was rapid in distilled and slow in saline.

Stuart et al. (2016) found that seawater inhibits formation while river water enhances it. Chlorine was also studied and found to enhance formation, however, these results are complicated by the fact that there may have been a competing reaction of chlorohydrin formation that induced the appearance of enhanced adipocere formation. The different results were suggested to have developed due to the bacterial activity in each environment. Elemental analysis was also carried out which showed that only seawater significantly affects the elemental composition and the authors suggested that the differences could be used to determine in what type of environment a body has been immersed.

3.1.4. Bacteria

Ueland et al. (2014) investigated lake environments in relation to the effect of aquatic bacteria in early adipocere formation. This was carried out with pigs and involved collection of bacteria samples from both the water and tissue. It was discovered that the presence of Gram-positive bacteria is essential in early formation as they provide the means to break down the tissue and release fatty acids through lipolysis. Most Gram-negative bacteria are not capable of this. At later stages, Gram-negative bacteria exclude Gram-positive bacteria.

Tissue submerged in deionised water, acting as the control, did not develop adipocere and bacterial concentrations of the lake tissue declined as adipocere formed.

3.1.5. Skin slippage

Skin slippage occurs during the decomposition process and is caused by the epidermal layer of the skin separating from the dermal layer and sloughing off the body (Wilson-Taylor, 2013).

Direct exposure of adipose tissue to water via skin slippage was shown to promote adipocere formation in rabbits (Widya, Moffatt & Simmons, 2012). This was also seen by Ueland et. al (2014). Skin slippage is used as a variable in PMI estimations indicating that this is a potential factor to explore in improving estimates (Widya et al., 2012).

3.1.6. Clothing/material

The presence of clothing is generally considered to enhance adipocere formation (O'Brien & Kuehner, 2007). Dix (1987), however, observed that clothes inhibited formation as areas with absent or loose clothing formed more advanced adipocere. The type of material in this case may have played a role in restricting formation as different types of material e.g. synthetic vs. natural fibres are known to have different effects.

In a personal observation by Guarreschi and Magni in a case of a suicide by hanging in a wood, adipocere was present exclusively in the feet of the victim found wearing shoes (Figure 3.1).

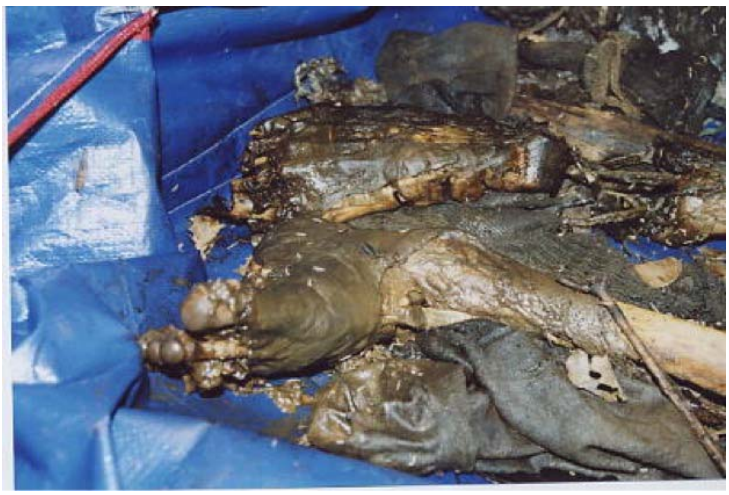

Figure 3.1 Feet of the victim of a suicide in a wood. Adipocere was present only on the feet of the victim, who was found wearing shoes (personal case of Edda Guarreschi and Paola Magni)

Notter and Stuart (2012) studied several materials in which a body may commonly be covered or wrapped. Polyester, wool clothing, cotton clothing, wool carpet, and acrylic carpet were investigated. All samples enhanced formation relative to the control sample. The wool and cotton materials made of natural fibre were better at enhancing formation than the synthetic polyester and acrylic carpet materials. Formation to an advanced stage of adipocere was faster in the natural fibre material. The wool carpet and the cotton clothing were the most efficient, followed by the wool clothing. The acrylic carpet enhanced formation better than polyester. The possible reasons given for the better formation with natural fibre material were the thickness of the material and water absorbency. Natural fibres are more absorbent than synthetic and the thicker wool carpet was better at enhancing formation than the wool clothing, possibly through absorbency or an insulating effect.

The effect of plastic on adipocere formation was less clear. Pakosh and Rodgers (2009) placed dismembered pig limbs into plastic bags and submerged them along with uncovered controls. The uncovered limbs lost soft tissue significantly more than the plastic enclosed, however, adipocere rarely formed within the plastic. This may have been due to low temperatures, low

submersion time or low fat content as the plastic was expected to enable adipocere to form. The limbs were preserved by the plastic but did not form adipocere.

3.2. Soil environments

Soil is composed of various minerals, salts, organic components and micro- and macroscopic organisms which all affect decomposition. When soil is removed from the ground and replaced during the burial of a body the nature of the soil changes. Compared to undisturbed soil, there is greater porosity and permeability of the soil. The passage of water and gases into the soil is also disrupted. The decomposition environment becomes anaerobic quite quickly and oxygen diffusion into the soil is thought to be blocked by gases from decomposition diffusing out of the soil (Dent et al., 2004). Burial in soil also restricts insects and scavengers in contrast to bodies deposited on the soil surface (Carter, Yellowlees & Tibbett, 2007). These features create an environment conducive to adipocere formation. Factors that inhibit or enhance adipocere formation in soil environments are listed in Table 3.2.

3.2.1. Soil type

Many soil types have been tested and shown to promote adipocere formation. These include loam-rich, clay-rich, sandy loam, clay, clay-gravel, sterilised loamy sand, and loamy sand. Soils that have been found to inhibit formation are those with low clay content and high organic matter (Durães et al., 2010; Forbes, Dent & Stuart, 2005). In other cases, it is unclear whether soils such as sand and silty sand promote or inhibit formation. These are both well-draining soils and would not be expected to maintain much moisture (Schotsmans, Van de Voorde, De Winne & Wilson, 2011). Adipocere is not likely to form in a dry, desert sand environment but

may form with burial in a sandy beach where there is constant moisture (Forbes et al., 2005). Fiedler and Graw (2003) considered the parent material the most important characteristic of soil whereas Durães et al. (2010) state that soil type is an indirect influence and the more crucial factor is the property of the soil, specifically the water content. Soils that retain moisture will generally promote adipocere formation. These soils would have poor drainage or be largely composed of fine particles (Fiedler & Graw, 2003). Strong, lateral water flow caused by soil such as clay also lead to high moisture (Fiedler et al., 2012).

3.2.2. Soil characteristics

Forbes et al. (2005a) studied the effects of soil pH, temperature, moisture and oxygen content. They found that a mildly alkaline pH, warm temperatures, anaerobic conditions and both dry and wet soil promoted adipocere. The optimal pH was between 5 and 9 and the optimal temperature was between 22-40˚C. At lower temperatures, formation will be inhibited while higher temperatures may promote desiccation and both inhibit bacterial growth. Excess moisture was not necessary but enhanced the rate of formation. Lime, cold temperatures and aerobic conditions inhibited adipocere formation. Acidic pH was also found to inhibit formation but other researchers have found that acidic soils were conducive to formation. Schotsmans et al. (2011) found extensive adipocere on a body buried in a well-draining soil with a pH between 2.6 to 4. Other inhibiting soil characteristics are biologically and microbially active soils (Fiedler et al., 2009).

3.2.3. Method of burial

The method of burial can range from direct burial into the soil, burial within a coffin, burial within a mass grave and burial with or without clothing or material covering the body (Dent et al., 2004; Evans, 1963; Forbes et al., 2005b).

Mass graves more commonly form adipocere than isolated, single graves (Evans, 1963). If the grave contains bodies piled on top of each other the layers tend to trap moisture and the middle of the grave will tend to contain bodies with the most adipocere formation. Bodies in mass graves that form only a single layer will exhibit decomposition closer to bodies that are buried in isolated, individual graves (Connor, 2009). Vane and Trick (2005) carried out research on a foot and mouth mass grave filled with pigs and cows and found that there was a mixture of early and late stage adipocere. At a depth of 50-70cm adipocere was found to be at an advanced stage but at 220cm samples were only observed to be in the early stage of formation. Different parts of the mass graves may have been isolated by infill resulting in a varied moisture content in each section. This would result in a mixture of early and advanced stage adipocere.

Forbes et al. (2005b) found that direct burial in soil, clothing, and plastic combined with clothing promoted adipocere formation whereas burial in plastic and coffins were found to inhibit formation. Polyester clothing allowed cation exchange which is thought to have allowed hardening of the adipocere to take place. Coffins inhibit formation due to slight aerobic conditions inside and will also prevent cation exchange compared to direct soil burial. Oak, lead and zinc coffins enhance formation while pine and spruce cause rapid decomposition along with coffins lined with a straw bedding (Fiedler & Graw, 2003).

The depth of burial may also have an effect on adipocere formation due to differences between insect and scavenger activity on the soil surface and beneath the soil. A body that is buried at a depth of 90cm or below is generally safe from scavengers and will be exposed to a reduced number of insects and microorganisms (Fiedler & Graw, 2003).

Weather may also have an influence as Evans (1963) observed that fog and misty conditions enhanced formation when they were present at the time of burial.

Table 3.2 Factors that enhance and inhibit adipocere formation in soil environments

Factor	Enhance	Inhibit	Author (year)
Temperature	Warm 20-40C	Cold More than 40C	Forbes et al. (2005a)
Scavengers	Absent	Present	Carter et al. (2007); Fiedler and Graw (2003)
Clothing/body coverings	Present Polyester	Absent Plastic coverings	Forbes et al. (2005b); Schotsmans et al. (2011)
Soil type	Loam-rich Clay-rich Sandy loam Loamy sand Clay Clay gravel Sand Sterilised Silty sand Poor draining Fine particles	High organic matter Gravel Active soil Low clay content	Durães et al. (2010); Fiedler and Graw (2003); Fiedler et al. (2009); Forbes et al. 2005
Moisture	High Excess Lateral water flow Fog and mist at time of burial	Low	Evans (1963); Fiedler et al. (2012); Forbes et al. (2005a)
Oxygen	Low, anaerobic	High, aerobic	Forbes et al. (2005a); Schotsmans et al. (2011)
Method of burial	Directly in soil Mass grave Oak, lead and zinc coffins Clothing with plastic Depth at or below 90cm	Pine and spruce coffins Straw bedding Wood shavings Lime Coffin	Dent et al. (2004); Evans (1963); Fiedler and Graw (2003) Forbes et al. (2005b); Green (2006); Schotsmans et al. (2011)
Soil pH	Mildly alkaline pH 5-9	Acidic Highly alkaline	Forbes et al. (2005a)

3.3. Dry environments

Development of adipocere was once thought to require a damp or moist environment, however, cases of adipocere in dry environments have been reported. Adipocere formation may also be found in combination with desiccation in this environment (Schotsmans et al., 2011). Research was carried out by Evans (1963) on bodies in dry vaults where over half of the bodies in each vault had formed extensive adipocere. This suggested that the internal water of the bodies was sufficient for formation and abundant moisture was not necessary.

Green (2006) also studied bodies contained in vaults consisting of an outer wooden shell and a sealed lead coffin with inner untreated wooden lining inside. Inside, the bodies were laid on wood shavings. In earth burials, wood shavings or sawdust enhance decomposition, however, the airtight condition of the coffins was assumed to produce sufficient conditions to promote adipocere.

Zimmermann, Laskay and Jackson (2008) described a case where suspected adipocere was found on a dry, indoor concrete surface. The body had been removed from the spot where it had been laying for several weeks and a stain had formed on the concrete surface. Conditions within the room were unclear but the conditions were thought to have been warm enough for bacterial activity as the body was quite decomposed when it was removed.

Nushida et al. (2008) described a case of a female that was sealed in a clothes box covered in plastic bags for 4 years. Adipocere formed extensively throughout the body, within the subcutaneous and the visceral fat.

4. Timing and distribution of adipocere within the body

The general description of adipocere varies considerably and may be observed as a yellow, greyish white, red, grey or grey-green substance and has a characteristic odour that has been likened to cheese and ammonia (Ubelaker & Zarenko, 2011; Pinheiro, 2006). Adipocere may form on a small area of the body or cover the body entirely (Kasuda et al., 2016). It was thought that only the subcutaneous tissues formed adipocere, however internal organs and bone marrow cavities can exhibit adipocere formation if adequate fat is present. Even in areas with low fat content, the pressure from putrefactive gases can cause liquefied fat to penetrate the tissue (Evans, 1963).

Just as formation varies with the environment a body is in, decomposition and adipocere formation vary between different bodies and even different areas of the same body. Bereuter and Lorbeer et al. (1996) cited a case of a man and woman in the same car submerged in a mountain lake. The man was skeletonised while the woman's soft tissue was preserved by adipocere. This was thought to be due to the different fat composition between men and women as women generally have a higher fat content than men. Ferreira and Cunha (2013) looked at 25 cases in a cemetery that all had a similar PMI of approximately 5 years but were observed to be in different stages of decomposition. Desiccation and adipocere have also been shown to occur on the same body under dry conditions where there is little external water present. A male buried in a shallow grave with his right leg and arm and temporal bone exposed exhibited desiccation in the exposed areas and adipocere in the areas covered by soil (Schotsmans et al., 2011).

4.1. Timing of formation

Formation of adipocere is most commonly observed at three to six months with partial formation at six weeks. Extensive formation takes at least a year and complete formation takes around 2 years (Bereuter & Lorbeer et al., 1996; Thali et al., 2011). Initial development within cutaneous areas of the body occurs after a couple of months and muscles will start transformation a least 6 months after the initial transformation process begins (Thali et al., 2011).

Several cases in which formation has been very rapid are described below. These cases all took place in areas of India (e.g. Delhi) which typically have a very hot and humid climate.

Several cases reported by Sikary and Murty (2015) involved formation in less than 2 days. Most of the bodies were located inside without air conditioning. These bodies all had roughly equivalent extent of formation between them. Several bodies were also recovered in water and showed a more variable extent of adipocere formation. Mohan Kumar et al. (2009) observed formation on a body within 3 days of submersion in a marshy pond and Powell (1917) observed adipocere formation around 4 days in a male buried in gravel and shale with heavy rainfall before and during the period of burial.

A body is rarely observed to be completely converted into adipocere, involving both the subcutaneous tissue and the internal organs, but a recently a case of complete transformation was reported in a male after 7 years of immersion within a car at the bottom of a lake. The authors used this to attempt to explain the process of complete adipocere formation (Kasuda et al., 2016). Submersion at the bottom of the lake with the car relatively undamaged was thought to have created a restrictive environment with low pH. There is little or no water flow at the bottom of a lake and this is thought to have contributed to maintaining a low pH and

may have inhibited formation of adipocere causing a slow conversion of the body. Absence of oxygen at the bottom of lakes creates desirable conditions for anaerobic bacterial growth. Oligotrophic conditions found in this environment also contribute to complete formation as well as the lack of scavengers.

Cotton et al. (1987) presented a similar case of extensive subcutaneous adipocere but without transformation of the organs. Two bodies were submerged in a vehicle in fresh water for 5 years. They were thought to have entered the water when the temperature of the water was at its highest point. The temperature subsequently dropped and this may have slowed down formation.

4.2. Subcutaneous tissue

Adipocere formation is initiated within the subcutaneous tissue and spreads outwards. The most common areas of formation are the cheeks, buttocks, abdomen and breasts. Adipocere is not likely to form on the hands or feet due to their relatively low fat content. Hands and feet tend to skeletonise and become disarticulated especially in water environments. An increase in the weight due to formation of adipocere may be experienced as well as a general increase in the size of a body (Cascio & Teodoro, 2014; Fiedler & Graw, 2003). Adipocere is generally more common in women, newborn babies, obese and well-nourished bodies due to their higher fat composition (Byard, 2016). There has been some correlation between age and formation of adipocere but not all studies support this (Evans, 1963; Sikary & Murty, 2015; Fiedler & Graw, 2003).

Although adipocere causes problems with PMI estimation it may also help investigations by preserving perimortem injuries or identifying features of the body in the subcutaneous tissues

and/or organs (Gupta & Jain, 2011; Mohan Kumar et al., 2009). Toxicology may also be able to detect chemicals, such as toluene, within a body that has been converted to adipocere (Inoue et al., 1996; Tanaka et al., 2016).

4.3. Organs

Organs may be found to have been protected from putrefaction simply due to the anaerobic environment produced by complete or nearly complete conversion of the subcutaneous fat or they may themselves be converted into adipocere (Cotton et al., 1987). Preservation of organs depends on the level of putrefaction at which adipocere begins and organs that have been well preserved indicate that formation occurred closely after death in a suitable environment. Organs are commonly found in a dehydrated form when adipocere covers their surface (Schotsmans et al., 2011).

Different organs will have different rates of decomposition and some will be preserved better than others. The intestines and spleen are quickly putrefied followed by the brain. The heart is more resistant followed by the kidney, lungs and bladder. The prostate and uterus are very resistant to putrefaction (Pinheiro, 2006).

The brain is the organ that is least expected to be preserved by adipocere (O'Connor et al., 2011; Papageorgopoulou et al., 2011). Only two cases of adipocere formation in the brain have been confirmed through chemical analysis. In other cases, chemical analysis of the brains was not carried out or it was unclear if it was carried out. Most cases of suspected adipocere formation in brains have been observed in archaeological or historical contexts with cold temperatures and acidic soil (O'Connor et al., 2011; Serrulla et al., 2016; Serrulla, Etxeberria, Herrasti, Cascallana & Olmo, 2017; Papageorgopoulou et al., 2011).

O'Connor (2011) pointed out that lipids are a component in brain tissue but mostly in the form of phospholipids or glycolipids. Triglycerides and free fatty acids constitute only a tiny portion of total lipid content. They also noted that all reported brains were shrunken, whereas adipocere usually increases the volume of the body.

Some of the most well-preserved adipocere brains were those found in Spanish Civil War mass graves (Serulla et al., 2016; Serulla, Etxeberria, Herrasti, Cascallana & del Olmo, 2017). The bodies were laid side by side and the bodies with adipocere were spread throughout the area. The gross morphology of the brains was present but could not be recognised histologically. It was proposed that the formation of adipocere in the brains was due to the gunshot wounds to the head which caused blood loss and inhibited brain decomposition. The climate at the time of burial was colder than usual with heavy rainfall which would lead to waterlogged soil. The wounds allowed water to come into contact with the brain and adipocere formed.

The other confirmed case of adipocere by Papageorgopoulou (2011) involved the preservation of the left cerebral hemisphere of a 13th century infant. The main brain structures were well preserved and could be identified macroscopically and microscopically. The body was situated in a leather envelope within a wooden coffin buried in acidic, clay soil that contained fresh and salt water. Continuous water immersion inside the grave was thought to have aided adipocere formation. The analysis carried out on this brain was only qualitative and it is difficult to determine to what extent adipocere conserved the brain. Adipocere may have only partially played a role in preservation. It is possible that a red-brown deposit on the surface may have been adipocere but it was not analysed (O'Connor et al., 2011).

Serrulla, Etxeberria and Herrasti (2015) found that within the same Spanish Civil War mass grave as described above, a heart was also found that had been conserved by adipocere.

Observations from more recent cases show that transformation of organs to adipocere is associated with long periods of immersion or burial. Adipocere formation was observed in the brain and bone marrow of a male submerged in a well for around 8 months (Magni, Borrini & Dadour, 2013). Burial of 6 years resulted in the conversion of the brain and heart along with subcutaneous tissue (Tanaka, Sato & Kasai, 2016). As described previously a male submerged in a car for 7 years had the entire subcutaneous and internal organs converted into adipocere (Kasuda et al., 2016). In other cases of long submersion, there may be extensive soft tissue preservation but the organs are not converted into adipocere.

4.4. Bones

Even when a body has been skeletonised, adipocere may be found on the bone surfaces and within the bone marrow cavities (Delabarde, Keyser, Tracqui, Charabidze & Ludes, 2013; Henderson, King, Caffell & Allen, 2015). Pokines and Higgs (2016) found adipocere in 20% of cases where bones were recovered in or near the ocean. The adipocere was found adhered to the bone and embedded in exposed cancellous bone. Adipocere within bones is protected from surface erosion and can persist for some time.

Moses (2012) studied defleshed bovine bones buried in artificial soil which developed adipocere on the surface of the bone. This showed that a large amount of adipose tissue may not be necessary for formation to occur. Laboratory experiments on the decomposition of bone found soft adipocere in bone marrow cavities submerged in pH 4 and pH 7 water while hard adipocere formed within the marrow cavities and surface of the bone in pH 10 water (Christensen & Myers, 2011).

Jopp-van Well et al. (2016) states that adipocere formation within bone is common in conventional burials. They present a case of an almost completely skeletonised corpse with extensive adipocere in the femur and humerus medullary cavities. The authors attempted to determine the PMI using radiocarbon dating in conjunction with knowledge on adipocere formation within bones. A possible period of death was determined as AD 1667 – AD 1952 through radiocarbon dating. The maximum PMI may be determined by macroscopic and microscopic identification of adipocere located throughout the bone but there is limited literature and the observations used are mostly accidental rather than from controlled experiments. In this case study of adipocere was not useful in further narrowing down the PMI estimated using radiocarbon dating. There is limited knowledge on bone adipocere formation and the PMI cannot currently be reliably estimated.

5. Case studies

This section focuses on case studies involving adipocere. These are particularly useful for forensic investigators as a comparison to current cases. Findings of a previous case may be able to be used as a reference for a current case if the cases took place in an area with a similar climate and geographical features.

Case studies are presented in Table 5.1 with the details of each case categorised into case year, PMI, body characteristics (sex, age, weight), adipocere location, the environment the bodies were recovered in, presence of clothing, presence of insects and cause of death.

The case years that were reported ranged from 1950 to 2014. The PMI ranged from 21 hours up to an estimated 30-150 years. The age range was between 4 years and 78 years with the majority of the cases involving males. Most bodies exhibited subcutaneous formation of adipocere while only one showed complete adipocere transformation where both subcutaneous and internal organs were transformed into adipocere. Cases involving water, soil and dry environments are all presented with the majority of cases found in water environments. Most bodies recovered from water were determined to have drowned, however in several cases death was due to gunshot injuries or sharp or blunt force trauma. Most bodies were clothed and only six cases reported the presence of insects.

Table 5.1 Case studies involving adipocere formation

Case Year	1973	1973	__	1983	1983	2002	__	__	__	__	__
PMI	11 months	10 months	6 months	5 years	5 years	30-100 years	25 days	27 days	30 days	31 days	36 days
Sex/Age/ Weight	M, 20	F, 22	F	M	F	M, 30-45	M, 24	M, 27	M, 37	M, 23	F, 24
Adipocere location	Subcutaneous, cheeks, abdominal wall, thighs	Subcutaneous	Subcutaneous	Extensive subcutaneous, face, neck, trunk, extremities	Extensive subcutaneous, face, neck, trunk, extremities	Femur tubules	Limbs	Limbs and buttocks	Limbs and abdominal wall	Limbs and abdominal wall	Limbs, buttocks, abdominal wall
Environment	Water (fresh), cold, silt-prone sinkhole (South Australia)	Water (fresh), cold, silt-prone sinkhole (South Australia)	Soil (Sicily, Italy)	Water (lake, in vehicle), water temp.= >21˚ C at immersion (Minnesota)	Water (lake, in vehicle), water temp.= >21˚ C at immersion (Minnesota)	Water (lake), depth = 32 metres (Austria)	Water (sea), average water temperature = 11.07˚˚ C (Adriatic Sea)	Water (sea), average water temperature = 14.5˚ ˚C (Adriatic Sea)	Water (sea), average water temperature = 15.4˚˚ C (Adriatic Sea)	Water (sea), average water temperature = 11.7˚ ˚C (Adriatic Sea)	Water (sea), average water temperature = 14.5˚˚ C (Adriatic Sea)
Clothing (Y/N)	Y (full-length wetsuit)	Y (full-length wetsuit with buoyancy vest)	N	Unknown	Unknown	N	Y (fully)	Y (heavily)	Y (socks and shoes)	Y (wetsuit)	Y (underwear)
Presence of insects (Y/N)	N	N	N	N	N	N	N	N	N	N	N
Cause of death	Drowning	Drowning	Head trauma, blunt instrument	Unknown	Unknown	Unknown (skull fracture present)	Drowning	Drowning	Drowning	Drowning	Drowning
Notes	Internal organs putrefied	Internal organs putrefied	Dismembered, wrapped in black plastic bags, internal organs dehydrated	Skeletal muscles desiccated, organs well-preserved grossly but not histologically	Skeletal muscles desiccated, organs well-preserved grossly but not histologically	Present as skeletal remains only	Floating close to land	Floating in high seas	Floating close to land	Floating close to land	Floating close to land
Author (year)	Byard (2009)	Byard (2009)	Cascio and Teodoro (2014)	Cotton et al. (1987)	Cotton et al. (1987)	Darok et al. (2005)	De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)

—	—	—	2010	—	—	—	—	—	1995	1995	1995
58 days	64 days	118 days	6 months to 2 years	4 months	6 months	10 months	34 days	n.d	30 days	120 days	570 days
M, 38	M, 54	M, 42	M, YA	F, 50	M, 22	F, 50	M	F, 40-50	Unknown	Unknown	Unknown
Saponification	Saponification	Total saponification	Subcutaneous (pelvic girdle)	Slight formation	Slight to moderate formation	Marked formation over entire body	Subcutaneous, scalp	Extensive subcutaneous	Unknown	Unknown	Unknown
Water (sea), average water temperature = 10.8°° C (Adriatic Sea)	Water (sea), average water temperature = 15°° C (Adriatic Sea)	Water (sea), average water temperature = 11.8° °C (Adriatic Sea)	Bank of river (Strasbourg)	Water (lake) (Missouri)	Water (lake) (Missouri)	Water (lake) (Missouri)	Water (sea, tropholytic zone) (Mediterranean Sea)	Water (pond) (Delhi)	Water (fresh) (British Columbia)	Water (fresh) (British Columbia)	Water (fresh) (British Columbia)
Y (light)	Y (partial)	Y (heavily)	N	N	Y (fully)	Y (fully)	Y (flight suit)	N	Unknown	Unknown	Y
N	N	N	Y (*Muscina stabulan,* 2 empty pupae)	N	N	N	Y (larva)	N	N	N	N
Drowning	Drowning	Drowning	Drowning	Unknown	Gunshot wounds to head	Head trauma, blunt instrument	Helicopter crash	Cut throat	Unknown	Unknown	Unknown
Floating in high seas	Recovered on beach	Floating close to land	Incomplete skeleton, diazepam and nordiazepam detected	Weighted down with concrete blocks	Lesser degree of adipocere formation on areas covered by clothing	Lesser degree of adipocere formation on areas covered by clothing		Wrapped in plastic bag	Skin sloughing also present	Skin sloughing also present	
De Donno et al. (2014)	De Donno et al. (2014)	De Donno et al. (2014)	Delabarde et al. (2013)	Dix (1987)	Dix (1987)	Dix (1987)	Dumser and Türkay (2008)	Gupta and Jain (2011)	Hobischak and Anderson (1999)	Hobischak and Anderson (1999)	Hobischak and Anderson (1999)

1996	1996	1994	—	—	—	2011	1998	1950	2008	2010	2007
91 days	180 days	3 months	13 months	Unknown (assessment of adipocere: 30-150 years)	7 years	3 years	4 years	6 years	8 months	5-7 months	65-66 hours
Unknown	Unknown	M, 24, 67kg	M, 78	M, 30-50	M, 50s, 52.3kg	M, 35	M, 55	M, 40s	M	M	M, 35
Unknown	Unknown	Subcutaneous, skeletal muscles	Complete subcutaneous	Medullary cavities of femur and humerus, compact and cancellous bone	Complete subcutaneous, muscles and internal organs	Right palm	Complete subcutaneous	Complete subcutaneous	Brain, bone marrow, left foot	Pelvis and lower limbs	Cheeks, front and back of trunk, upper limbs except hands, lower limbs
Water (fresh) (British Columbia)	Water (fresh) (British Columbia)	Water (river, within vehicle)	Water (river)	Tideland of river (River Elbe, Otterndorf)	Water (lake), depth = 40 metres, water temp. = 25˚˚ C at time of immersion	Soil (loamy), depth = 1.8 metres (Warsaw, Poland)	Soil, depth = 4 metres	Water (fresh) (Lake Orta, Italy)	Water (well, ground water) (Piedmont, Italy)	Water (well, ground water) (Sicily, Italy)	Water (marshy pond)
Y	Unknown	Unknown	Unknown	N	Unknown	N	Unknown	Y	Y (clothing and shoes)	Y (trousers)	Y
N	Y (*Hydrotaea Robineau-Desvoidy, Piophilidae,Creophilus, Necrodes*)	N	N	N	N	N	N	N	Y (Calliphoridae, Fanniidae, Muscidae, Trichoceridae, Sphaerocerdidae, Psychodidae, Nematocera)	Y (Syrphidae, Muscidae, Cleridae)	N
Unknown	Unknown	Drowning	Drowning	Unknown	Unknown	Strangulation	Gunshots to head	Gunshots to head	Drowning	Unknown	Gunshot injuries
	Skull and lower mandible on sand, rest of body found in pool of water	Toluene was detected within adipocere	Adipocere visualised using multislice computed tomography	Skull and partial thorax	Complete adipocere formation	Left hand uncovered, right hand gloved, right palm and finger ridges preserved		Skin and subcutaneous tissue rapidly falling off body after retrieval		Entomological evidence collected 20 months after body recovery	
Hobischak and Anderson (1999)	Hobischak and Anderson (1999)	Inoue et al. (1996)	Jackowski et al. (2005)	Jopp-van Well et al. (2016)	Kasuda et al. (2016)	Klemczak et al. (2015)	Kobayashi et al. (1999)	Lentino (1956)	Magni et al. 2013	Magni et al. 2013	Mohan Kumar et al. (2009)

—	—	—	—	2013	2013	2013	2013	2013	2013	2013	2013
4 months	5 months	4 years	7 months	1 day 20 hours	2 days 7 hours	1 day 15 hours	3 days 5 hours	1 day 17 hours	1 day 4 hours	3 days 5 hours	2 days 3 hours
M, 41	M, 42, 45kg	F, 77	M, 65, 50.4kg	M, 42	M, 35	F, 59	F, 5	M, 47	M, 55	M, 37	M, 4
Subcutaneous, upper body	Marked formation, brain	Almost complete subcutaneous and visceral	Subcutaneous, head, neck, right shoulder, upper torso, left leg	Front and back of trunk, upper limbs except hands	Front of trunk, upper limbs except hands	Face, front of trunk, upper limbs, lower limbs	Face, front and back of trunk, upper limbs, lower limbs	Abdomen, upper limbs, lower limbs except feet	Front and back of trunk, upper limbs except hands, lower limbs except feet	Front of trunk, sides of back, arms	Front of trunk, upper limbs except hands
Soil (graveyard), depth = 85 centimetres	Water (sea)	Dry, clothes box covered with plastic bags	Soil (sandy), depth = 30-42 centimetres, pH 2.6-4 (Belgium)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)
Y (short-sleeved shirt, trousers)	Y	Unknown	N	Y	Y	Y	Y	Y	Y	Y	Y
N	N	N	Y (*Calliphora vicina,* larvae and pupae)	N	N	N	N	N	N	N	N
Gunshot to abdomen	Drowning	Unknown	Smothering	Hanging	Unknown	Unknown	Drowning	Alcohol intoxication	Drowning	Septicaemia from burn	Unknown
Buried above an old grave, head buried under cement, lower body skeletonised	Nicotine and cotinine detected	Detected novel fatty acid of adipocere: *cis*-12-octadecenoic acid	Differential decomposition (desiccation, adipocere)								
Mohd Nor and Das (2012)	Nishimura et al. (2009)	Nushida et al. (2008)	Schotsmans et al. (2011)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)

2013	2013	2013	2014	2014	2014	2014	2014	—	—	—
2 days 9 hours	1 week	3 days 3 hours	6 days	3 days	21 hours	3 days	6 days	>6 months	>6 months	5-6 years
M, 38	M, 14	M, 33	F, 24	M, 35	M, 13	F, 29	M, 45	F, 20-30	M. >60	M, 20s
Abdomen, chest, arms	Face, front/back of trunk, upper limbs except hands, lower limbs except feet	Back of trunk, right upper limb except hand, front of right thigh	Face, front and back of trunk, upper limbs, lower limbs	Front of trunk, upper limbs except hands, front of lower limbs except feet	Abdomen, lower back, left upper limb except hand	Face, left side of trunk, lower back, arms, lower limbs	Front of trunk, left side of back, upper right thigh	Base of skull	Right side of skull, scalp, fractured ends of hyoid	Subcutaneous except head and extremities, brain, heart, muscles
Water (river bank, hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Water (river bank, hot, humid conditions) (South Delhi)	Dry (closed box inside shop, hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Dry (hot, humid conditions) (South Delhi)	Soil	Soil	Soil, depth = 1 metre
Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y
N	N	N	N	N	N	N	N	N	N	N
Drowning	Drowning	Head injury	Multiple stab wounds	Drowning	Strangulation	Cut throat	Unknown	Strangulation	Strangulation	Cardiac injury
								Only skull present with fragments of hyoid bone embedded in adipocere	Skull, long bone fragments and decomposed tissue present	Toluene detected at high levels, unclear what effects this had on death
Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	Sikary and Murty (2015)	S. Smith (1939)	S. Smith (1942)	Tanaka et al. (2016)

n.d = not determined; F = female; M = male; YA = young adult

6. Discussion

Knowledge pertaining to adipocere has been gathered for several hundred years and, as stated by Ubelaker and Zarenko (2011), there has been much insight into the various aspects of adipocere formation.

Adipocere formation and the timing of that formation is highly variable throughout the body and is influenced by a wide variety of factors both environmental and body specific. Development of reference material outlining the timing and distribution of adipocere formation throughout the body may help forensic professionals during investigations. This includes the different environments in which adipocere may be formed and the variables within them that may have influenced adipocere formation e.g. temperature, presence of insects and pH level as well as variables pertaining to the body e.g. sex, age, weight and presence of clothing. Findings will better apply to a case if it occurs in a similar climate and the area of the case has similar geographical features to the reported case studies.

Study of the elemental composition of adipocere may aid in further distinguishing adipocere samples into the different stages of formation, early, intermediate and late stage, and may also be useful to differentiate between water environments in which adipocere has formed. Chemical analysis of the environment in which adipocere forms is also necessary to further understand the variables, such as pH, bacteria and water flow, that influence formation (Stuart et al., 2016).

The key to adipocere's persistence has been cited as the absence of oxygen. In the presence of oxygen, adipocere will degrade and decomposition of the remains will continue (Schoenen & Schoenen, 2013). An additional pathway of adipocere degradation under poikiloaerobic conditions has been proposed (Fiedler et al., 2015). Further research in the area of adipocere

degradation is needed. Areas of focus that have been suggested are the biochemical pathways and environmental influences that govern adipocere formation as well as bacteria associated with adipocere formation and degradation. DNA analysis of bacteria found in association with adipocere could be utilised to determine species and their relative abundance (Schoenen & Schoenen, 2013; Ueland et al., 2014).

The estimation of post-mortem interval (PMI) is still complicated by the presence of adipocere and no reliable method has yet been developed to address the problem. Skin slippage was observed to enhance the formation of adipocere in rabbits (Widya et al., 2012) and as skin slippage is used in PMI estimates this is a potential factor that, with further research, may improve PMI estimates.

7. Conclusions

Extensive research has been conducted on the phenomenon of adipocere formation on decomposing remains. The general chemical composition of adipocere is well-established and has been used to carry out research into the mechanism of adipocere formation as well as attempting to solve the problem of post-mortem interval estimation.

The estimation of the post-mortem interval in adipocerous bodies remains difficult and continued research into the chemistry of adipocere and factors affecting adipocere formation may aid in getting closer to establishing a reliable model for post-mortem interval estimation associated with the presence of adipocere.

Another area of much-needed research is around adipocere degradation. It is known that degradation requires an aerobic environment and may be facilitated by macrofauna but the environmental factors affecting degradation and the involvement of microorganisms is not well established.

Further study into these areas above will assist in a better understanding of the nature of adipocere and this will assist with death investigations involving adipocere formation, especially if PMI estimation techniques can be improved through this research.

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