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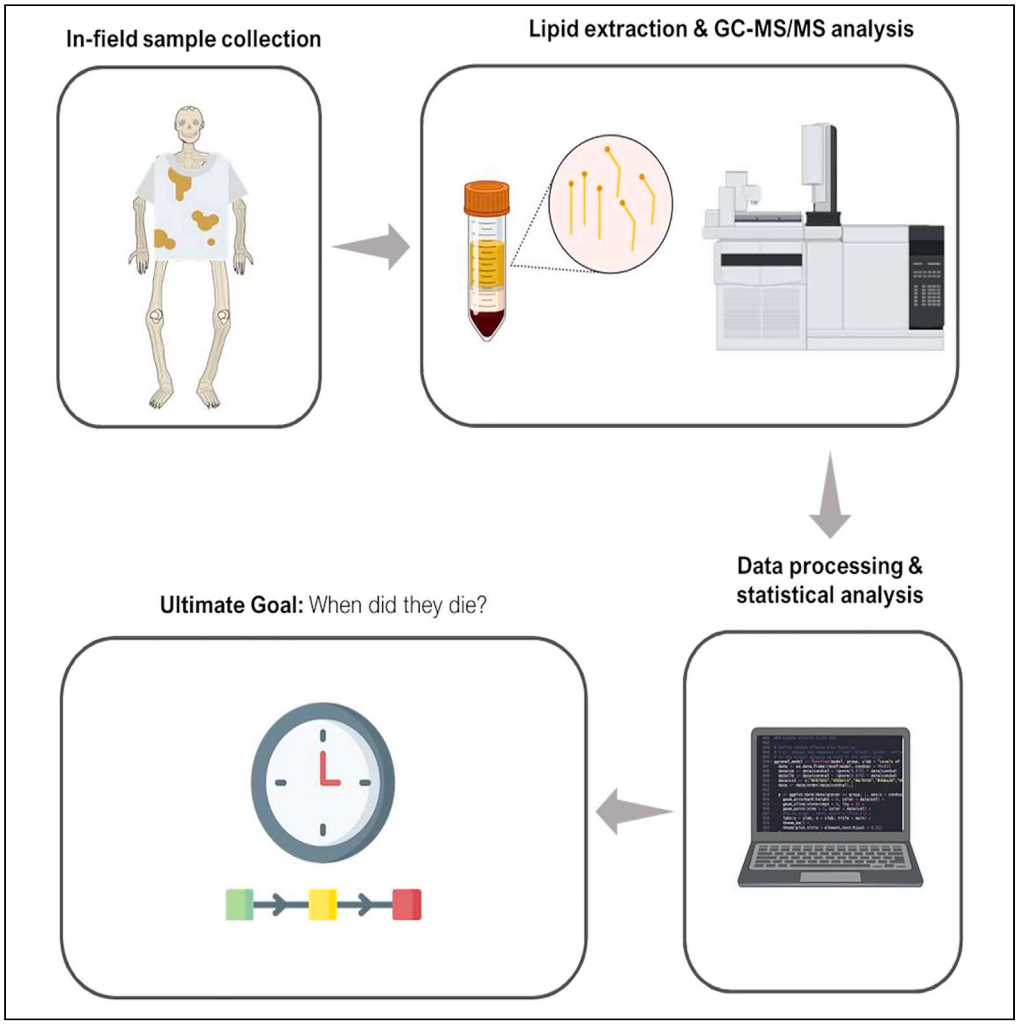
The use of generalized linear mixed models to investigate postmortem lipids in textiles



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Highlights

Textiles serve as an excellent host for capturing postmortem lipids

Results indicate oleic and palmitic acids to be most suitable lipids for future work

Future application could aid cases where remains are absent, scavenged, or relocated

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The use of generalized linear mixed models to investigate postmortem lipids in textiles

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SUMMARY

Human remains are oftentimes located with textile materials, making them a ubiquitous source of physical evidence. Human remains are also frequently discovered in outdoor environments, increasing the exposure to scavenging activity and soft-tissue decomposition. In such cases, postmortem interval (PMI) estimations can be challenging for investigators when attempting to use traditional methods for reconstructive purposes. Lipid analysis is an emerging area of research in forensic taphonomy, with recent works demonstrating success with the detection and monitoring of lipids over time. In this work, generalized linear mixed models (GLMMs) were utilized to perform rigorous statistical analyses on 30 lipid outcomes in combination with accumulated-degree-days (ADD). The results of this study were consistent with recent works, indicating oleic and palmitic acids to be the most suitable lipids in textiles to target for future use as soft-tissue biomarkers of human decomposition. Interspecies differences between humans and pigs were also addressed in this work.

INTRODUCTION

The overall rate, manner, and progression of human decomposition is highly complex and dependent on a range of intrinsic and extrinsic variables. Because of this, post-mortem interval estimations continue to be a major challenge for forensic investigators when faced with the discovery of human remains. After death, the body undergoes a series of semi-predictable stages: fresh, bloat, active, advanced, and skeletal.[1] However, the time that elapses between these stages can be unpredictable and often non-linear,[2] making it very difficult to estimate the post-mortem interval.

Data obtained from both forensic casework and experimental research[3–6] have documented temperature to be one of the main extrinsic covariates influencing the rate and manner of decomposition. Work by Megyesi et al. in 2005[7] demonstrated that 80% of the variation within decomposition can be accounted for by accumulated-degree-days (ADD). The authors of that study outlined that the remaining variation include other climatic and contextual factors like scavenging activity, peri- and postmortem injuries, rainfall, and sunlight.[7] ADD are units that represent the accumulation of thermal energy in a chronological combination of time and energy.[7] In this manner, it is said that when equal units of thermal energy (ADD) are applied to remains, a relatively equal amount of decomposition is to be expected.[8] This is because climate affects the time expected to elapse during each decomposition stage, and thus determines the overall rate at which the remains will decay.[3,5] Therefore, the ability to assess the impact of ADD on measurable outcomes of decomposition holds great potential for elucidating clarity regarding the problem of determining the post-mortem interval.

Postmortem lipid analysis is a promising new area of research in forensic taphonomy, with several recent works[9–12] demonstrating great success with the use of clothing as a host to capture these decomposition lipids. As the human body contains approximately 15% of adipose tissues,[13] during the decomposition process, the lipids contained within these tissues are released into the immediate environment. Hence, by analyzing the patterns in these postmortem lipids, in conjunction with ADD, it is possible to use lipids as soft-tissue biomarkers of decomposition and to aid in possible PMI estimations.

This idea was recently put forward by the authors of the current study, who successfully developed a method for the extraction and targeted analysis of postmortem lipids in textiles using gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS).[12] That work provided results which related the

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cumulative relative abundance of 30 lipids, to three broad decomposition categories; "early," "middle," and "late". While that research was fundamental in providing a foundation for future work, the results of Collins et al.[12] were mostly descriptive in nature, making it difficult to discern which of the lipids were most appropriate for investigation as soft-tissue biomarkers of decomposition.

The current work investigates the impact of ADD, as the unit of measurement for chronological time, on postmortem lipids in clothing, as the measurable outcome of decomposition, using GC-MS/MS. Furthermore, this work uses generalized linear mixed models (GLMMs), containing both fixed and random effects where the latter is used to account for heterogeneity between individuals, to perform rigorous statistical analyses on the 30 lipid outcomes in combination with ADD. For the purpose of sample size requirements, the current study supplemented four pig cadavers, in addition to two human donors. Pigs have often been utilized and widely accepted in taphonomic research due to anecdotal similarities to humans with respect to skin, digestive, and immunological systems,[14–16] in addition to the natural limitations that come with working with human remains. As such, this study also provides a unique research opportunity to further investigate the interspecies differences between pigs and humans recently addressed by Collins et al.[11] The overall aim of the current work was to assess which of the 30 lipids could be suitable for use as soft-tissue biomarkers of human decomposition contained in textiles to aid PMI estimations in future casework.

RESULTS AND DISCUSSION

Visual decomposition

The visual stages of decomposition for all six individuals have been described in detail.[12] To summarize, the visual decomposition observed for the pigs and humans was considerably different between the summer and winter trials. During summer, Human 1 (H1), Pig 1 (P1), and Pig 2 (P2) were exposed to periods of heavy rainfall, with an overall mean temperature of 19.4°C. H1 underwent changes from the fresh stage to the bloat stage within the first ten days post-placement and entered into an advanced state of mummification without a distinctive active stage. In contrast, P1 and P2 progressed from fresh to bloat and then into active decay within the first ten days post-placement, while later reaching full skeletonization by day 35 post-placement.

The winter donors Human 2 (H2), Pig (P3), and Pig (P4) experienced less rainfall and a lower overall mean temperature of 14.8°C. As a result, all three individuals of the winter trial desiccated by day 42 post-placement, with less distinctive differences in the visual stages of decomposition between species compared to the summer trial.

GLMMs

Model selection was performed manually and began with running the full model proposed in the *Data processing and statistical analysis* section. Backward selection was then performed by removing non-significant fixed effects (using a nominal significance level of 5%) and checking the residuals. Also, in some cases analysis of variance (ANOVA) was used to determine whether it was beneficial to switch to a purely fixed effect only model or not i.e., removing the random effect from the model. The point estimates and corresponding 95% confidence intervals of the fixed effects for all selected GLMMs are reported in Table S2. For the analytes where a mixed effect model was used, point estimates and confidence intervals for the random intercept variance parameter, $\sigma^2_{k,}$ are also displayed in Table S2. The point estimates of fixed effects for the selected models are presented in the matrix plot (Figure 1).

Of the 30 lipids analyzed, the binary indicator for human was not statistically significant for 10 lipids, cholesterol, coprostanol, deoxycholic acid, 5α-cholestanone, hexacosanoic acid, lithocholic acid, palmitoleic acid, stigmasterol, β-sitosterol, and 25-hydroxycholesterol (Table S2). That is, for these 10 lipids, there was no statistically clear difference between pigs or humans in terms of the (log mean) lipid readings.

Of the above 10 lipids, the models for cholesterol, 5α-cholestanone, and palmitoleic acid were of most interest, particularly when considering a forensic research perspective where lipid analysis (irrespective of species) is the goal. These three analytes were all found to exhibit a quadratic relationship with the covariate (ADD) (Figure 1). Specifically, as ADD increased over time the lipid value also increased before the rate of increase slowed and eventually the analyte values plateaued by the end of the study. This finding was consistent with the fact that, during the earlier stages of decomposition prior to the release of fluids into the environment, many zero readings are recorded for the lipids during this period (Figure S1). Afterward,

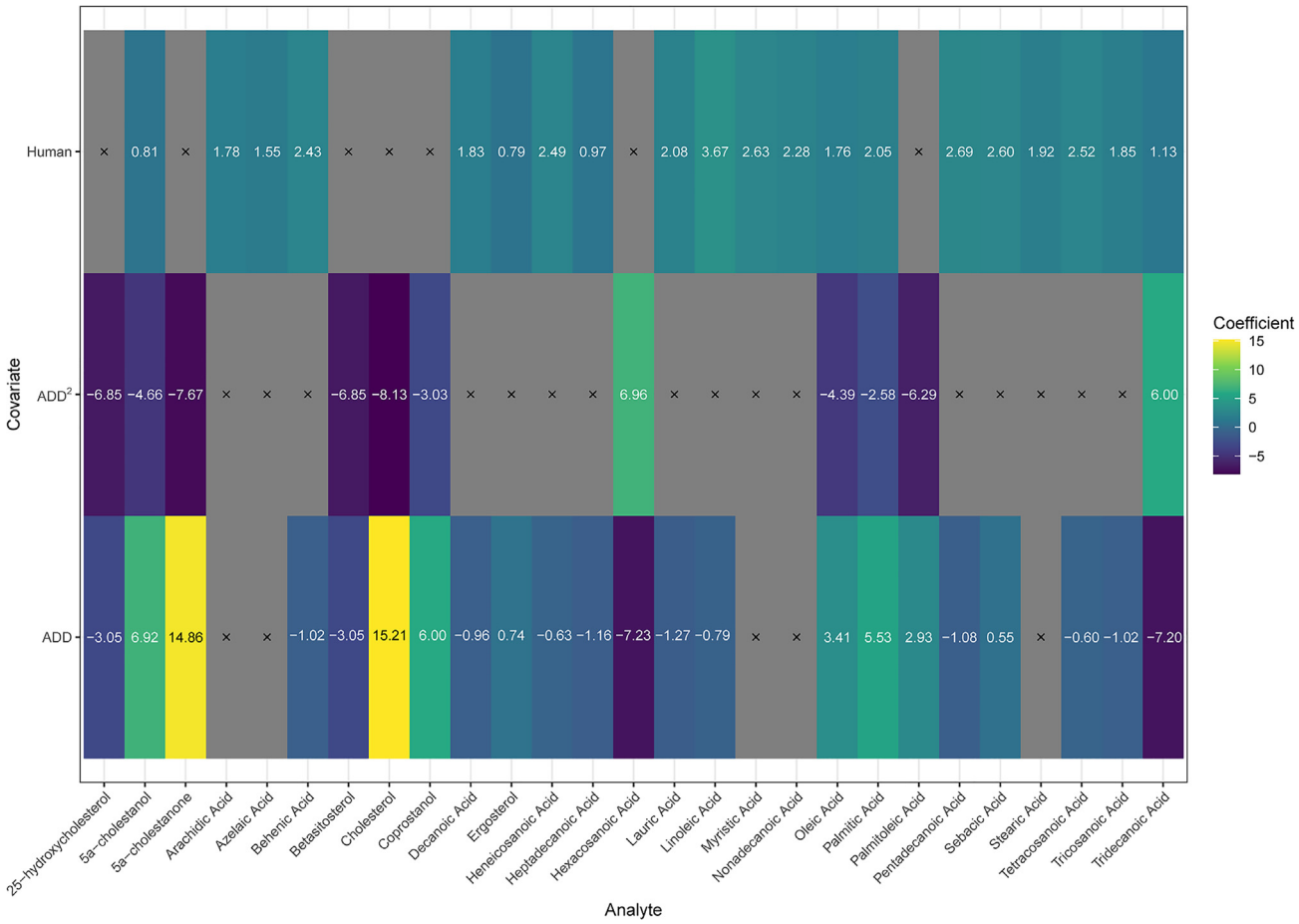

Figure 1. Matrix plot displaying the estimated fixed effects for the models fitted to the analytes

x indicates the corresponding covariate was not included in the selected model. Yellow indicates the covariate had a high positive effect on the analyte in question while dark blue indicates the covariate had a high negative impact.

once the fluids are released from the body, there is a sudden influx recorded for the outcomes. This influx then plateaus, reaching a point of saturation despite the continuum of ADD.

As discussed by Collins et al.,[12] cholesterol is a tissue-derived sterol in higher animals,[17,18] however it is also ubiquitously found in soil environments due to plant and fungal contributions.[19,20] In animals, cholesterol plays an integral part in lipid metabolism as well as being a structural component of plasma membranes.[21,22] During the processes of decomposition, cholesterol is known to reduce into its stanol derivatives, 5α-cholestanol and coprostanol,[23] with 5α-cholestanone being an intermediary of this reduction process.[24,25] The difficulty with targeting sterols in decomposition studies lies in the background contribution of environmental sterols from plants, animals and fungi.[26–28] Thus, some caution should be employed when examining cholesterol in isolation for certain studies; instead, researchers should also consider targeting its degradation stanone intermediary, 5α-cholestanone.

On the other hand, palmitoleic acid is one of the most abundant unsaturated fatty acids in human serum and tissues[29] and has also been well documented to be a component in the decomposition by-product, adipocere.[30,31] Adipocere is a gray, wax-like substance that is formed by the anaerobic bacterial hydrolysis of lipids.[32,33] Thus, in context of the results from the model, it appears that palmitoleic acid can be used to track the formation of adipocere during decomposition. In addition, due to the quadratic relationship exhibited between ADD and ADD2 for the model of palmitoleic acid, it is clear that adipocere formation follows the aforementioned trend whereby this product is formed; it accumulates and then eventually

dissipates to some degree over time thus, providing further insight to the decomposition timeline by the appearance and timing of these decomposition by-products.

The model for cholesterol included fixed effects only (Table S2), suggesting that the (baseline) readings for this analyte were relatively similar across the individuals, while the models for palmitoleic acid and 5α-cholestanone included both fixed and random effects, suggesting more evidence of heterogeneity across the individuals (Table S2). Considering this, cholesterol, 5α-cholestanone, and palmitoleic acid provide great potential for future use as soft-tissue biomarkers of decomposition with studies that involve both pigs and humans.

The binary indicator for human showed a statistically significant and positive effect for the remaining 20 lipids (Figure 1), meaning that there was evidence to suggest that all 20 of these lipids could potentially be used to differentiate between pigs and humans. However, the particular models for each analyte must be further considered in context of the available decomposition literature in order to extract any meaningful conclusions. Several of these 20 lipids can be traced to sources other than soft-tissue decomposition e.g., dietary (i.e., heptadecanoic acid[34]) or environmental (i.e., ergosterol[35]) factors. Although some of these lipids may provide some useful insight to the decomposition ecology as a whole, the aim of the current study was to identify lipids that could be suitable as soft-tissue biomarkers of decomposition, therefore, originating primarily from the soft-tissues.

With this in mind, the models for oleic acid and palmitic acid were the most interesting (Figure 1). Both of these lipids, which are known decomposition by-products,[30,31] ended up requiring the full GLMM outlined in in the *Data processing and statistical analysis* section (Table S2). Like cholesterol, 5α-cholestanol, and palmitoleic acid, the models for oleic acid and palmitic acid also established a clear quadratic relationship between the log mean of the outcome and the covariate. This trend directly reflects the dynamicity of the decomposition process itself, whereby lipids are released from the body and absorbed by the textiles as a result of active decay, and slowly reduce over time as the remains enter into the advanced and skeletal stages of decomposition. This again offers evidence that these particular lipids could be suitable for further investigation as soft-tissue biomarkers of human decomposition.

It is important to recognize that some of the heterogeneity observed for several of the analytes, namely myristic acid, oleic acid, palmitic acid, and palmitoleic acid was influenced by some extreme observations for H1, as shown in the scatterplots (Figure S2) and random effects plots (Figure S3). While it is conceivable that the placement season,[3–6] being summer for H1 and winter for H2, could have impacted on these results, it is just as likely that systematic differences could have driven this. As such, it must be acknowledged that H1 may be responsible for driving the strong positive effect for the binary indicator of human group for some of these analytes.

Overall, the results of this study provide a foundation for the future of decomposition lipid analysis as success was achieved, which could relate ADD, as the chronological measurement of time, to lipids in textiles, as the measurable outcomes of decomposition. In addition, the results provided further insight to the interspecies differences addressed by Collins et al.,[11] with particular mention to the lipids related to the formation of adipocere. Based on the preliminary findings yielded in this study, textiles serve as an excellent host for postmortem lipids. In addition, these results indicated that oleic acid and palmitic acid would be the most suitable lipids for further investigation for use as soft-tissue biomarkers of human decomposition and PMI indicators. Although the study presented results obtained from lipids in textiles, it is possible that the approach could be applied to other matrices that host decomposition lipids, such as tissue or soil. Future application of such methods presents as particularly useful for complex cases where remains are absent, have been clandestinely relocated or scavenged, and investigators are needing to rely solely on alternative measures for PMI estimations and event reconstruction.

Limitations of the study

In order to fully reach meaningful conclusions, the authors recommend future work to include several more donors, with samples obtained over many years. This type of longitudinal analysis would allow for the detection of changes in the lipid characteristics at both the group and individual level, which would provide understanding about the efficacy and stability of these lipids as potential biomarkers. With a larger dataset, it could be possible to make predictions of ADD, given the measurable lipid outcomes and further investigate the influence of other factors such as sex and age of the donors.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Field site
  - Sample collection
  - GC-MS/MS sample preparation
  - GC-MS/MS analysis
  - Data processing and statistical analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107371.

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AUTHOR CONTRIBUTIONS

Conceptualization: S.C., M.U., and B.S.

Methodology: S.C., L.M., F.K.C.H., M.U., and B.S.

Validation: S.C., L.M., and F.K.C.H., Formal analysis: S.C. and L.M.

Investigation: S.C., M.U., and B.S.

Supervision: M.U., B.S., and L.M.

Writing—original draft: S.C.

Writing—review & editing: S.C., L.M., F.K.C.H., B.S., and M.U.

DECLARATION OF INTERESTS

Authors declare that they have no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
HPLC-grade acetone	Sigma Aldrich, Australia	CAS#: 67-64-1
HPLC-grade chloroform	ChemSupply, Australia	CAS#: 67-66-3
HPLC-grade acetonitrile	Labscan Ltd., Australia	CAS#: 75-05-8
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMCS)	PM Separations, Australia	CAS#: 25561-30-2
Stearic acid-d3	Merck, Australia	CAS#: 62163-39-7
Cholesterol-d7	Sapphire Bioscience, Australia	CAS#: 83199-47-7
25-hydroxycholesterol	Sigma-Aldrich, United States	CAS#: 2140-46-7
5α-cholestanol	Sigma-Aldrich, United States	CAS#: 80-97-7
5α-cholestanone	Sigma-Aldrich, United States	CAS#: 481-21-0
Arachidic Acid	Sigma-Aldrich, United States	CAS#: 506-30-9
Azelaic Acid	Merck, Australia	CAS#: 123-99-9
Behenic Acid	Sigma-Aldrich, United States	CAS#: 112-85-6
Cholesterol	Sigma-Aldrich, United States	CAS#: 57-88-5
Coprostanol	Sigma-Aldrich, United States	CAS#: 360-68-9
Decanoic Acid	Sigma-Aldrich, United States	CAS#: 334-48-5
Deoxycholic Acid	Sigma-Aldrich, United States	CAS#: 83-44-3
Ergosterol	Sigma-Aldrich, United States	CAS#: 57-87-4
Heneicosanoic Acid	Sigma-Aldrich, United States	CAS#: 2363-71-5
Heptadecanoic Acid	Sigma-Aldrich, United States	CAS#: 506-12-7
Hexacosanoic Acid	Sigma-Aldrich, United States	CAS#: 506-46-7
Lauric Acid	European Pharmacopeia, France	CAS#: 143-07-7
Linoleic Acid	Sigma-Aldrich, United States	CAS#: 60-33-3
Lithocholic Acid	Sigma-Aldrich, United States	CAS#: 434-13-9
Myristic Acid	Sigma-Aldrich, United States	CAS#: 544-63-8
Nonadecanoic Acid	Sigma-Aldrich, United States	CAS#: 646-30-0
Oleic Acid	Sigma-Aldrich, United States	CAS#: 112-80-1
Palmitic Acid	Sigma-Aldrich, United States	CAS#: 57-10-3
Palmitoleic Acid	Sigma-Aldrich, United States	CAS#: 373-49-9
Pentadecanoic Acid	Sigma-Aldrich, United States	CAS#: 1002-84-2
Sebacic Acid	Sigma-Aldrich, United States	CAS#: 111-20-6
Stearic Acid	Honeywell, Australia	CAS#: 57-11-4
Stigmasterol	Sigma-Aldrich, United States	CAS#: 83-48-7
Tetracosanoic Acid	Sigma-Aldrich, United States	CAS#: 557-59-5
Tricosanoic Acid	Sigma-Aldrich, United States	CAS#: 2433-96-7
Tridecanoic Acid	Sigma-Aldrich, United States	CAS#: 638-53-9
β-sitosterol	Sigma-Aldrich, United States	CAS#: 83-46-5
GCMS-TQ8030 Triple Quadrupole Gas Chromatograph	Shimadzu, Kyoto, Japan	N/A
AOC-20i auto injector	Shimadzu, Kyoto, Japan	N/A
HP-5MS (30 m × 0.250 mm) column	Agilent Technologies, Australia	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
R	https://www.r-project.org/	4.2.2
ggplot2	https://ggplot2.tidyverse.org/	3.4.1
glmmTMB	https://cran.r-project.org/web/packages/glmmTMB/index.html	1.1.6
DHARMa	https://cran.r-project.org/web/packages/DHARMa/index.html	0.4.6
Other		
HOBO Weather Station	OneTemp, Marleston	N/A
HOBO® U30 No Remote Communication (NRC) data logger	OneTemp, Marleston	N/A
100% white cotton t-shirt	Anko, Kmart	N/A
0.2 µm hydrophobic PTFE syringe filters	MicroAnalytix, Australia	N/A
250 µL polymer inserts	Agilent Technologies, Australia	N/A

RESOURCE AVAILABILITY

Lead contact

If you have a request for more information or resources related to this paper, please contact Maiken Ueland (maiken.ueland@uts.edu.au).

Materials availability

No new materials were created during this study.

Data and code availability

- All data are available in the main text or the supplemental information.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available form the lead contact upon request

EXPERIMENTAL MODEL AND SUBJECT DETAILS

A total of two human and four pig cadavers were utilized in this study. The human donors were received through the UTS Body Donation Program, with consent provided in accordance with the New South Wales Anatomy Act (1977). Ethics approval was provided by the UTS Human Research Ethics Committee (UTS HREC NO. ETH18-2999). The study consisted of two trials: Trial 1 commenced during the Australian summer on January 29, 2021 and included one human donor (H1) and two pigs (P1 and P2). Trial 2 began in the Australian winter on June 11, 2021, and also included one human donor (H2) and two pigs (P3 and P4). The cause of death for H1 was Alzheimer's disease and lung cancer for H2. The cause of death to all pigs included in this study (P1, P2, P3, P4) was a bolt to the head. All individuals were clothed in a white 100% cotton t-shirt, as described in,[10] laid in a supine position on the soil surface at AFTER, and were allowed to decompose naturally. Anti-scavenging cages were used to allow natural entomological activity to progress, while preventing scavenging from local fauna. Donor information for all six individuals is summarized in Table S1.

METHOD DETAILS

Field site

This study was conducted at the Australian Facility for Taphonomic Experimental Research (AFTER), an open eucalypt woodland on the Cumberland Plain in Western Sydney, privately owned by the University of Technology, Sydney (UTS). Weather conditions at the field site were monitored using a HOBO Weather Station with a HOBO® U30 No Remote Communication (NRC) data logger (OneTemp, Marleston).

Sample collection

Textile samples were collected as outlined in.[11] In summary, textile samples were acquired in duplicates, at different time points, between day 0 and day 105 post-placement (Table S1) from the anterior surface of the remains, using sterilized scissors. Each textile sample was photographed *in-situ* prior to collection, individually packaged in paper envelopes and stored in a cooler box for transportation to the laboratory, where the samples were kept at −18°C when not in use. Each sample was dried in a fume cupboard at ambient temperature to inhibit bacterial and fungal growth, with adhering soil, hair and tissue removed.

GC-MS/MS sample preparation

Samples were prepared according to.[12] Specifically, 35 mg of textile matrix was weighed per sample. Afterward, 2 mL of HPLC-grade acetone (Sigma Aldrich, Sydney, Australia) and 2 mL of HPLC-grade chloroform (ChemSupply, Port Adelaide, Australia) were added to each vial and sonicated, without heat, for 20 minutes. The samples were then left at 4 °C for 16 hours for extraction. Afterward, 1 mL of the resulting extract was filtered through a 0.2 μm hydrophobic PTFE syringe filter (MicroAnalytix, Taren Point, Australia). Then, 25 μL of the filtered aliquots were spiked with 20 μL 10 ppm stearic acid-d3 (Merck, Macquarie Park, Australia) and 50 μL of 10 ppm cholesterol-d7 were added as internal standards. These were dried down under a gentle stream of nitrogen at 40°C. Samples were then reconstituted in 40 μL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMCS) (PM Separations, Capalaba, Australia) and 10 μL of HPLC acetonitrile (Labscan Ltd., Gillman, Australia), and heated for 30 minutes at 70°C prior to being transferred into 250 μL polymer inserts (Agilent Technologies, Mulgrave, Australia). All samples were extracted and aliquoted in duplicates and injected onto the GC-MS/MS, where duplicate runs were conducted, giving a total of four data points per sample with the addition of a full scan injection for quality control purposes.

GC-MS/MS analysis

Analysis was performed applying the method developed by[12] using a GCMS-TQ8030 Triple Quadrupole Gas Chromatograph fitted with an AOC-20i auto injector (Shimadzu, Kyoto, Japan). In detail, samples were injected in split mode at a temperature of 270°C onto an Agilent HP-5MS (30 m × 0.250 mm) column with a film thickness of 0.25 μm. The system was run in multiple reaction monitoring (MRM) mode as outlined in,[12] with a total run time of 18.75 minutes (Figure S4). A total of 30 lipids, containing a mixture of fatty acids and sterols, were selected for targeted analysis. In alphabetical order, these are: arachidic acid, azelaic acid, behenic acid, cholesterol, coprostanol, decanoic acid, deoxycholic acid, ergosterol, heneicosanoic acid, heptadecanoic acid, hexacosanoic acid, lauric acid, linoleic acid, lithocholic acid, myristic acid, nonadecanoic acid, oleic acid, palmitic acid, palmitoleic acid, pentadecanoic acid, sebacic acid, stearic acid, stigmasterol, tetracosanoic acid, tricosanoic acid, tridecanoic acid, β-sitosterol, 5α-cholestanol, 5α-cholestanone and 25-hydroxycholesterol.

Data processing and statistical analysis

The mean area results obtained from the GC-MS/MS MRMs were processed in accordance with.[12] In summary, the lipids were normalized to their respective internal standards; fatty acids to stearic acid-d3 and the sterols to cholesterol-d7. The normalized abundances were then subject to the exclusion criteria proposed in,[12] before further standardizing by dividing the grand population standard deviation, per lipid (i.e., individuals aggregated). ADD was calculated as outlined in,[7] and further standardized. As a covariate, ADD was also standardized by dividing by the cluster-specific standard deviation (i.e., by individual). These standardization procedures are commonly used to stabilize mixed-effects model fitting, and do not have any statistical influence on the final model conclusions.[36] The standardized outcomes and ADD values were plotted using R[37] via the package ggplot2[38] (Figure S2).

Generalized linear mixed models (GLMMs[39–41]) were used as the principal method of statistical analysis in the study. GLMMs offer the capacity to handle non-continuous and non-normally distributed responses, where the fixed effects are shared among all the individuals (thus representing population-level effects) while the random effects account for potential sources of heterogeneity across the six individuals (pigs and humans, thus representing individual-level effects). Put another way, the random effects are specific to a single individual, and are included to allow for the fact that analyte readings within an individual are likely to be more similar compared to readings between individuals i.e., the analyte readings within an individual are correlated over time.

In this study, a Tweedie distribution was used to model the response (analyte) in the GLMM, as this is well suited to data which are heavy-tailed and include an excess of zero values,[42] as is the case with many analytes used in the analysis (see Figure S1). GLMMs with Tweedie responses were fitted for each outcome in R[18] using the glmmTMB package.[43] In more detail, let y_{ijk} denote the reading of the kth analyte ($k = 1, \ldots, 30$) for the ith individual ($i = 1, \ldots, 6$) at the jth time point ($j = 1, \ldots, n$). Then the Tweedie-response GLMM is specified as follows:

$$[y_{ijk} \mid u_{ik}] \sim \text{Tweedie}\,(y_{ijk}|u_{ik}, \mu_{ijk}, p_k, \varphi_k),$$

$$\log(\mu_{ijk}) = \beta_{0k} + \text{ADD}_j\beta_{1k} + \text{ADD}_j^2\beta_{2k} + \text{Human}_i\beta_{3k} + u_{ik},\ u_{ik} \sim \textit{Normal}\,(0, \sigma^2{}_k),$$

where, conditionally on the random effect u_i, the analytes y_{ijk} are independent observations from a Tweedie distribution with mean $\mu_{ijk} = E[y_{ijk}|u_{ik}]$, power parameter $1 < p_k < 2$ and dispersion parameter $\varphi_k > 0$. Here, the fixed effects included in the GLMM are the intercept term, the standardized ADD value, its squared term (ADD^2), and the binary indicator of whether the ith individual was a human carcass or not (1 = human; 0 = pig). The squared effect of ADD, given by the inclusion of ADD2, was incorporated into the GLMMs as exploratory analysis suggested there was evidence for a quadratic relationship with ADD for several of the analytes. The random intercept ui was the only random effect included in the model. Residual analysis (Table S3) was performed using the DHARMa package,[44] which in tandem with exploratory data analysis was used to both check model assumptions and decide on the final form of the fixed and random effects structure to include. After fitting GLMMs separately to each analyte, the point estimates of the fixed effects from each model were used to construct a matrix plot in R[18] to visualize the results.