



The impact of environmental factors on the production of peptides in mammalian decomposition fluid in relation to the estimation of post-mortem interval: A summer/winter comparison in Western Australia



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ABSTRACT

We report the peptide content of decomposition fluid produced under field-based conditions and in the absence of a soil matrix. Sixteen domestic pig (*Sus scrofa domestica*) cadavers were used to model human decomposition in trials conducted in the summer and winter months in Western Australia. Physical characteristics were recorded and the peptide components of decomposition fluid were analysed using high performance liquid chromatography-time of flight mass spectrometry. A range of peptides was consistently detected in both summer and winter. Thirty seven peptides were common to both trials; 22 originating from haemoglobin subunit beta, 1 from haemoglobin subunit alpha, 4 from beta-enolase, and 2 from creatine kinase. In agreement with our previous findings, 13 peptides occurred consistently, regardless of trial conditions. Degradation patterns for haemoglobin subunits alpha and beta in summer and winter were similar when expressed in ADD and when adjusted for differences in temperature. The consistent identification of several protein-specific peptides generated during decomposition trials conducted under different temperature and rainfall regimes suggests that quantitative peptide analysis may be useful in estimating time since death.

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1. Introduction

Understanding the taphonomic processes associated with mammalian decomposition and how environmental variables affect the process is fundamental to the estimation of the post-mortem interval (PMI). An accurate PMI estimation can significantly contribute to an investigation involving unidentified human remains by eliminating potential decedents from a pool of possibilities, while at the same time, including others [1]. There are a number of intrinsic and extrinsic factors that impact upon the rate of decomposition, the most significant of which is the temperature to which the body is exposed [1–4]. Studies have found that more than 80% of the variability seen in soft tissue decomposition is due to temperature differentials [1,5,6], while

other environmental factors such as humidity and rainfall have a lesser impact [2]. Trauma, insect activity, moisture content, body size and clothing are other factors known to impact the decomposition process, however, there is much debate about how much a role these factors play [1,3,7,8].

Various methods are currently available for estimating PMI, however, not all methodologies are reliable [1]. Traditionally, forensic pathologists estimate PMI in the hours after death by assessing the classic triad: algor mortis, livor mortis, and rigor mortis [9]. Forensic entomology is a useful tool for estimating PMI in the later stages of decomposition, as insects are known to colonise remains at specific intervals and stages of decomposition [2,3,10,11]. Such techniques have been used with some success [12–16], however, they are not without their limitations [1,2,11].

The Total Body Score (TBS) method developed by Galloway et al. [17] and modified by Megyesi et al. [4] has been commonly used as a quantitative means to measure decomposition [4,7,18–20]. It has been suggested that when coupled with Accumulated Degree Days (ADD), the TBS scoring method can accurately determine PMI across varied environments by accounting for differences in

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temperature and by providing symmetry between individual experiments [3,4]. However, the model is not without limitations. Using the method on remains that are pre-adolescent, burned, buried or submerged is not advised [4]. Nawrocka et al. reported that the scores assigned by the model to remains in multiple stages of decomposition were inconsistent. Moreover, the same study found that indicators such as specific skin colour and vague descriptors such as “heavy maggot activity” also contributed to the inconsistencies [21]. Furthermore, several studies that have attempted to validate the method in different geographical locations found it either over or under estimated the PMI [22–24]. Studies have yet to validate the method in Western Australia, a State that, in summer, has attained maximum temperatures as high as 50.5°C [25]. Similar algorithm-based techniques have been reported in the literature [6,7], however, they have also proved unsuccessful in estimating PMI across different geographic regions [26].

In contrast, studies that have used biochemical approaches have relied upon more robust parameters to assess the rate of decomposition with encouraging results [15,27–33]. It is suggested that of the biochemical techniques used to estimate the PMI, measurement of the rate and extent of protein degradation is a promising approach [31,28–33]. Pittner et al. demonstrated that the degradation of several skeletal muscle proteins occurred with high reproducibility and at different time points during the decomposition process, allowing for more precise estimations of PMI during the intermediate and late postmortem stages [33]. Our previous work, although taking a different analytical approach, demonstrated that blood proteins in decomposition fluid degraded in a regular fashion in the presence or absence of insect activity [32]. These studies were conducted under controlled conditions in a laboratory-based environment, so the applicability of these findings, expressed in ADD, to a field-based forensic setting is yet to be demonstrated.

This study investigates the impact of temperature on the degradation patterns of proteins in mammalian decomposition fluid during the summer and winter months in a Western Australian environment. The relationship between ADD and the production of peptides is also explored.

2. Materials and methods

2.1. Research facilities

Decomposition studies were carried out at a bushland site located at Murdoch University, Western Australia (−32°03′ S, 115°50′ E), 16 km south of Perth CBD. Studies were conducted using a pre-existing caged structure, to restrict access to the carcasses by larger predators. The structure also provided shelter from the rain to minimise sample dilution and protection from direct sunlight to minimise evaporation. A data logger (EL-USB-2+) was used to record and monitor temperature and humidity every 10 min for the duration of the study; this was suspended above the carcasses. The first trial was conducted over summer (January–February 2018) and the second in winter (July–November 2018). The study was approved by the Murdoch University Animal Ethics Committee (Cadaver approval number: 376).

2.2. Cadavers

Domestic pigs (*Sus scrofa domesticus*) were utilised to model the human decomposition process. Domestic pigs are considered to be an appropriate substitute for humans in decomposition studies due to their similarity in weight, muscle to fat ratio, hair coverage, internal anatomy and gut fauna [34]. Eight adult female pigs (20

kg) were euthanised by bolt gun to the temple 2 h prior to commencing each study.

2.3. Decomposition trials and observations

Each carcass was placed on a raised, galvanised metal platform. Openings in the platform enabled the fluid to drain away from the body into a plastic collection container to ensure that the decomposition process was not influenced by fluid accumulation. The platforms on which the carcasses rested remained parallel to the ground to prevent decomposition fluid flowing to different areas within the carcass.

Collection containers were monitored daily and fluid samples collected between 0800 and 0900 for the first two weeks and, thereafter, every second day until the conclusion of each study (when carcasses reached mummification). The fluid was mixed thoroughly to ensure that a homogeneous mixture of the accumulated fluid was sampled on each monitored day. Five x2 mL fluid samples were collected from each carcass container using a 1 mL plastic pipette and placed in 2 mL graduated microtubes. Samples were stored at −80 °C until analysed. Any remaining fluid not sampled was discarded and containers were rinsed thoroughly with water before being replaced beneath each carcass.

Elapsed time was documented in accumulated degree days (ADD) to account for fluctuations in temperature [4]. Observations of physical appearance, including skin discolouration, bloating and deflation, skin ruptures, hair loss and bone exposure were recorded on each sampling day. Photographs were also taken to accompany the observations. The stage of decomposition was assessed and evaluated using the Total Body Score (TBS) method developed by Keough et al. [35].

2.4. Sample preparation and analysis

The full preparation and analysis of decomposition fluid samples were as described in Nolan et al. [32]. Fluid samples were diluted, filtered and loaded onto a Vivaspin 2 10,000 MWCO filter (Sartorius, Australia); this ensured that not only larger peptides were removed from the sample, but bacteria and enzymes responsible for peptide degradation were also eliminated. Samples were centrifuged for a minimum of 60 min and thereafter in 30-min increments until the volume of retentate was 100 µL. The filtrate was then loaded onto a pre-conditioned Strata-X 33 µm Polymeric Reverse Phase, 60 mg/3 mL column (Phenomenex, Australia), washed and eluted. The sample was then evaporated to dryness and stored at −80°C until analysed. The sample was reconstituted and analysed using a Shimadzu Prominence nano HPLC system (Shimadzu) coupled to a 5600 TripleTOF mass spectrometer (Sciex). Peptides were loaded onto an Agilent Zorbax 300SB-C18, 3.5 µm, 0.075 × 150 mm column (Agilent Technologies) and separated using a 40 min linear gradient of water/ acetonitrile/0.1% formic acid at a flow rate of 300 nL/min. Peptides were eluted through a nanospray interface into a 5600 TripleTOF mass spectrometer (Sciex). Spectral data was analysed using ProteinPilot™ 5.0 Software (Sciex) against the UniProt *Sus scrofa* database.

2.5. Data analysis

Peptide data were analysed using the VennDiagram package [36] in RStudio version 3.5.1 [37]. Peptide sequences were aligned using Geneious (v11.1.5), with the following alignment settings: Alignment type = Global alignment with free end gaps, Cost Matrix = Blosum90, Gap open penalty = 22, Gap extension penalty = 3, Refinement iterations = 2 [38].

3. Results and discussion

3.1. Decomposition fluid analysis

3.1.1. Summer trial

Decomposition fluid was collected, when available, from the eight domestic pig cadavers during a three-week monitored period. Average daily temperatures ranged from 20.75 to 27.5°C, with a maximum of 36.5° and a minimum of 15.5° recorded during the monitored period. At the conclusion of the trial, cadavers exhibited mummification of the head, neck, trunk and limbs; skin was brown and leathery in appearance and was adhered to the bone. An average total body score of 24 was recorded. Fluid was collected from cadavers 1, 3, 7 and 8 until day 7 (ADD 175), from cadavers 2 and 5 until day 9 (ADD 218), and cadavers 4 and 6 until day 10 (ADD 240). Analysis of the samples revealed the presence of numerous peptides in the decomposition fluid from each cadaver throughout the trial period. Strong correlations were found between ADD and total peptide counts ($r = -0.89$), as well as the presence of peptides and the presence of the proteins from which they were derived ($r = 0.94$) (Fig. 1).

3.1.2. Winter trial

Decomposition fluid was collected, when available, from eight domestic pig cadavers during a sixteen-week monitored period. Average daily temperatures ranged from 8.4 to 19.58°C, with a maximum of 29.0° and a minimum of 3.0° recorded during the monitoring period. At the conclusion of the trial, cadavers exhibited mummification of the head, neck, trunk and limbs. Skin was black, brown in appearance and dry to the touch (hair was still present). A small amount of bone exposure was seen in the face and limbs. An average total body score of 24 was recorded. Fluid was collected from cadaver 7 until day 22 (ADD 274), cadaver 2 until day 26 (ADD 327), cadavers 1, 3 and 5 until day 30 (ADD 385), cadaver 6 until day 32 (ADD 406), and cadavers 4 and 8 until day 34 (ADD 429). As with the summer trial, analysis of the samples revealed the presence of numerous peptides in the decomposition fluid from each cadaver throughout the trial period. Again, a strong correlation was observed between the peptides present and the presence of the specific proteins from which they were derived ($r = 0.84$). No clear correlations were found between ADD and total peptide counts ($r = 0.22$) (Fig. 2). Throughout the trial, decomposition fluid samples recovered from cadavers 4, 5 and 6 were heavily diluted by rain due to cadaver positioning within the caged structure. These were therefore excluded from any analyses.

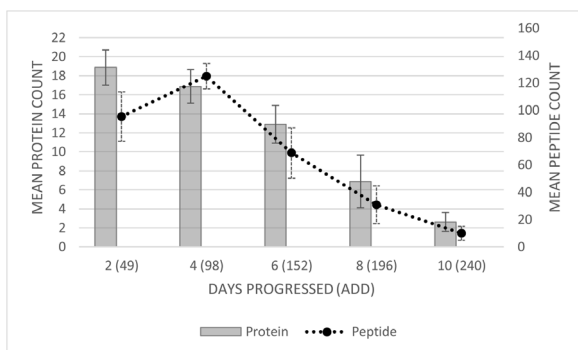


Fig. 1. Mean number of proteins (histograms) and peptides (line) (>95% CI) detected in decomposition fluid samples collected from Cadavers 1–8 on analysis days 2–10 (ADD 49–240) in Summer trial. Figure depicts correlations between ADD and total peptide counts in addition to total number of peptides and total number of proteins from which they were derived.

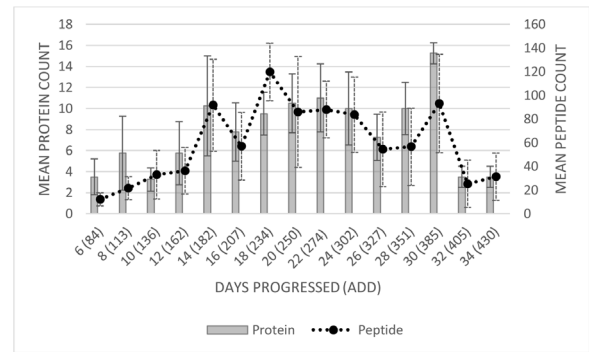


Fig. 2. Mean number of proteins (histograms) and peptides (line) (>95% CI) detected in decomposition fluid samples collected from Cadavers 1, 2, 3, 7 and 8 on analysis days 6–34 (ADD 84–429) in Winter trial. Figure depicts correlations between ADD and total peptide counts in addition to total number of peptides and total number of proteins from which they were derived.

3.2. Proteins

Proteins were classed as being present if they were identified more than 50% of the time over the course of each trial period (10 and 34 days for summer and winter, respectively) [39]. In both trials two proteins were present in all decomposition fluid samples, haemoglobin subunit unit alpha and haemoglobin subunit beta. Further investigation of the winter data set, focusing on proteins present $\geq 50\%$ of the time across the trial period or present in 3 out of 5 sample days, an additional four proteins, beta-enolase, creatine kinase, L-lactate dehydrogenase and troponin C2, were identified (Table 1). Due to the small data set generated by the summer trial, additional proteins, such as these, were not apparent.

Of the six proteins identified across both trials, five were detected in our previous study [32]: creatine kinase, beta-enolase, L-lactate dehydrogenase, haemoglobin subunit alpha, and haemoglobin subunit beta. None of these proteins correspond with the findings of Pittner et al. [31,33] (Fig. 3).

3.3. Targeted peptides

3.3.1. Mean peptide chain length

Correlation between chain length and days post-mortem was most apparent for peptides originating from haemoglobin subunit alpha ($r = 0.91$ and 0.69 for summer and winter, respectively). As time progressed, trends towards shorter peptides were apparent (Figs. 4a and 5a), in agreement with our previous findings [32]. In contrast, for troponin C2 a trend towards larger peptides was observed as time progressed ($r = 0.84$), however the relative standard deviations for the calculated means were large (21.2%) (Fig. 5f). Studies have shown that troponin is highly susceptible to proteolysis and is considered to be one of the most specific and sensitive markers for myocardial death [40,41]. Typically, the hydrolysis of troponin yields small peptides as cardiac death takes place [40], however this is not evident within the current study and is regarded as an anomalous finding. It is possible that the small sample size for troponin C2 in this study, adversely affected the results obtained. If a larger sample size had been available, results may have been similar to those previously published. Because of this anomaly, Troponin C2 was not considered further in the current study but its suitability as a marker for PMI estimation will be the subject of future investigations. Correlations between peptide chain length and time were less apparent for the remaining proteins (Figs. 4b–d and 5b–e) and again similar to

Table 1

Identification of the 37 peptides found to be common in decomposition fluid samples collected in summer and winter. Peptides marked with an * were previously reported by Nolan et al. [32].

| Protein | Peptide sequence |
|---------------------------|-----------------------|
| Haemoglobin subunit alpha | AAWGKVGQGAGAHGAEALER |
| | AVGHLDLPGAL |
| | FLGFPTTK* |
| | GHLDDLPGAL |
| | LSHGSDQVKAHQKQVADALTK |
| | VGGQAGAHGAEAL |
| | VGGQAGAHGAEALERM |
| | VGHLDLPGAL |
| | VLSAADKANVKAAWGK |
| | ESFGDLSNADAVMGPNK* |
| | ESFGDLSNADAVMGPNKVK* |
| | FESFGDLSNADAVMGPNK |
| | FESFGDLSNADAVMGPNKVK |
| | FGDLSNADAVMGPNK* |
| | FGDLSNADAVMGPNKVK* |
| | GDLSNADAVMGPNK |
| | GDLSNADAVMGPNKVK* |
| Haemoglobin subunit beta | GKVVNDEVGGEALGR |
| | GKVVNDEVGGEALGRL* |
| | GLWGKVVNDEVGGEALGR |
| | KVVNDEVGGEALGR |
| | KVVNDEVGGEALGRL* |
| | NVDEVGGEALGRL* |
| | SFGDLSNADAVMGPNK* |
| | VDEVGGEALGRL |
| | VHLSAEEKEAVL |
| | VHLSAEEKEAVLG |
| | VHLSAEEKEAVLGL |
| | VNVDEVGGEALGR |
| | VNVDEVGGEALGRL* |
| | WGKVVNDEVGGEALGR |
| | DLFDPPIQDR* |
| | KDLFDPPIQDR* |
| | Creatine kinase |
| IVGDDLTVTNPKR | |
| QIVGDDLTVTNPKR | |
| SGVNIQIVGDDLTVTNPKR | |
| Beta-enolase | |
| | |
| | |
| | |

those previously reported [32]. It is reasonable to assume, however, that average chain length is indicative of the extent of the degradative process occurring in the parent protein. For haemoglobin subunit alpha, it appears that degradation is still occurring and, as a consequence, the average chain length continues to decrease with each sampling interval. For the other proteins, the lesser correlation between time and peptide chain length could indicate that degradation of the protein in question has ceased. However, if a protein is susceptible to degradation, a correlation between time and decreasing peptide chain length should still be evident in the initial stages of its hydrolysis. Proteins in this study for which the peptide chain lengths remained relatively constant and, hence, were poorly correlated with time, are not considered to be useful markers for PMI estimation.

3.3.2. Peptide mapping

Peptide sequences were mapped against reference sequences for haemoglobin subunit alpha (XP_020942625) [42], haemoglobin subunit beta (NP_001138313) [43], beta-enolase (NP_001037992) [44] and creatine kinase (NP_00112342) [45] to identify the average degradative patterns on each sample day. Degradation patterns for haemoglobin subunit alpha and haemoglobin subunit beta were consistent with those previously reported [32]. Peptides generated from haemoglobin subunit alpha were derived, progressively, from the amino terminal end to the carboxy terminal end of the protein as time progressed (Fig. 6a–g). The most abundant peptides generated in the winter

trial on day 6 (ADD 84) were derived from residues 4–25 (Fig. 6a). However, in contrast to our previous study, the number of peptides derived from this region remained fairly constant as the trial progressed (Fig. 6a–d). Peptides derived from residues 60–80 on days 14–30 (ADD 182–385) were also prominent (Fig. 6b–d), but less so than in our previous study [32]. An additional region, not previously reported, from which peptides were derived, involved residues 110–120 on days 22–30 (ADD 234–385) (Fig. 6c–d). A comparison of the summer and winter trials revealed that peak abundancies were consistent between the two seasons (Fig. 6). The number of peptides derived from residues 4–25 was consistent throughout the trial as were peptides derived from residues 60–80 (Fig. 6e–f). However, peptides derived from residues 110–120 were more abundant in summer than in winter (Fig. 6). It is important to note that the degradation patterns for haemoglobin subunit alpha in summer and winter were similar when expressed in ADD and when differences in temperature were accounted for. Peak shapes do differ due to the differences in sample size between the two trials, however, the general trends observed are similar. This suggests that the degradative process is consistent over time.

In agreement with previous findings [32], haemoglobin subunit beta displayed a consistent degradative pattern throughout each trial (Fig. 7a–f). The most abundant peptides were derived from residues 15–35 on days 6–30 (ADD 84–385) (Fig. 7a–d) in winter and on days 2–6 (ADD 49–152) (Fig. 7e–f) in summer. Abundant peptides were also generated from residues 45–65 on days 14–30 (ADD 182–385) (Fig. 7b–d) in winter and on days 2–6 (ADD 49–152) (Fig. 7e–f) in summer. As with haemoglobin subunit alpha, when comparing data based on ADD, only minimal differences in degradation patterns were observed between summer and winter. This is a further indication that the degradative process remains consistent over time.

Degradation patterns for creatine kinase and beta-enolase have yet to be reported in the literature. Our previous findings suggested that the degradative patterns for these proteins were inconsistent when replicates were compared directly [32]. However, when peptide content was averaged in the current study, breakdown patterns were found to be uniform between both summer and winter. The most abundant peptides generated from creatine kinase degradation were derived from residues 50–65 on days 14–30 (ADD 182–385), and from residues 15–30, 80–130, and 370–380 from day 22 (ADD 234) onwards in winter (Figs. 8a–c). Though the summer trial yielded variable results, the most abundant peptides were still consistent with those detected in winter. The most abundant peptides were derived from residues 50–65 on days 2–6 (ADD 49–152); from residues 80–100 on days 2–10 (ADD 49–240) and from residues 370–380 from day 6 (ADD 152) onwards. Peptides generated from the degradation of beta-enolase displayed just one abundance peak, which was consistent between summer and winter. These peptides were derived from residues 310–330 on days 14–30 (ADD 182–385) in winter and on days 2–10 (ADD 49–240) in summer (Fig. 9a–f).

When the temperature differential between summer and winter is taken into account, the peptides associated with haemoglobin subunits alpha and beta, creatine kinase, and beta-enolase are consistent. These results are in agreement with our previous findings derived from decomposition trials conducted under controlled conditions [32]. The finding that protein-specific peptides are produced at different stages of the decomposition process suggests that the use of protein biomarkers in estimating the post-mortem interval may be more applicable than previously reported. Pittner et al. identified several protein products (not found in this study) which appeared to have potential for estimating PMI for up to 10 days post-mortem [31,33]. However, the current study has detected potential marker peptides in decomposition fluid well beyond this timeframe and this may

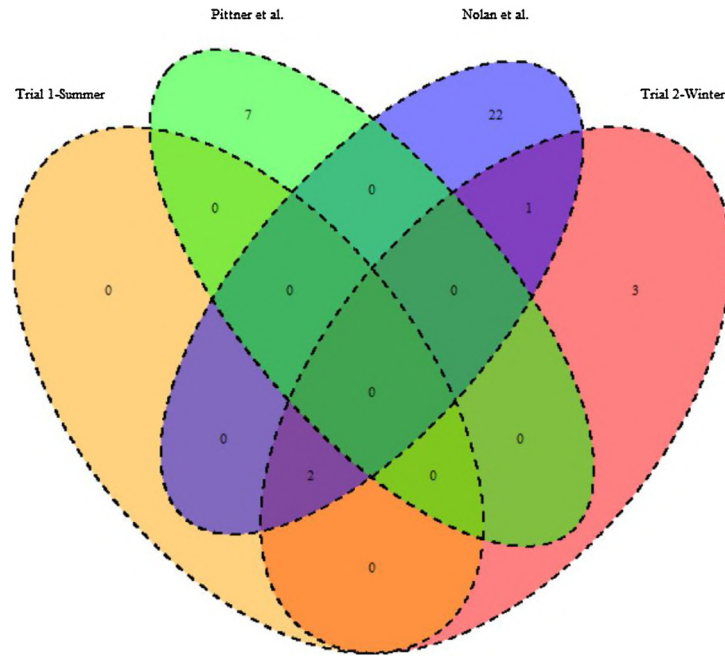


Fig. 3. Venn diagram reporting total proteins of interest in relation to the determination of PMI as reported by Pittner et al. [31,33], Nolan et al. [32], and this study (Summer and Winter).

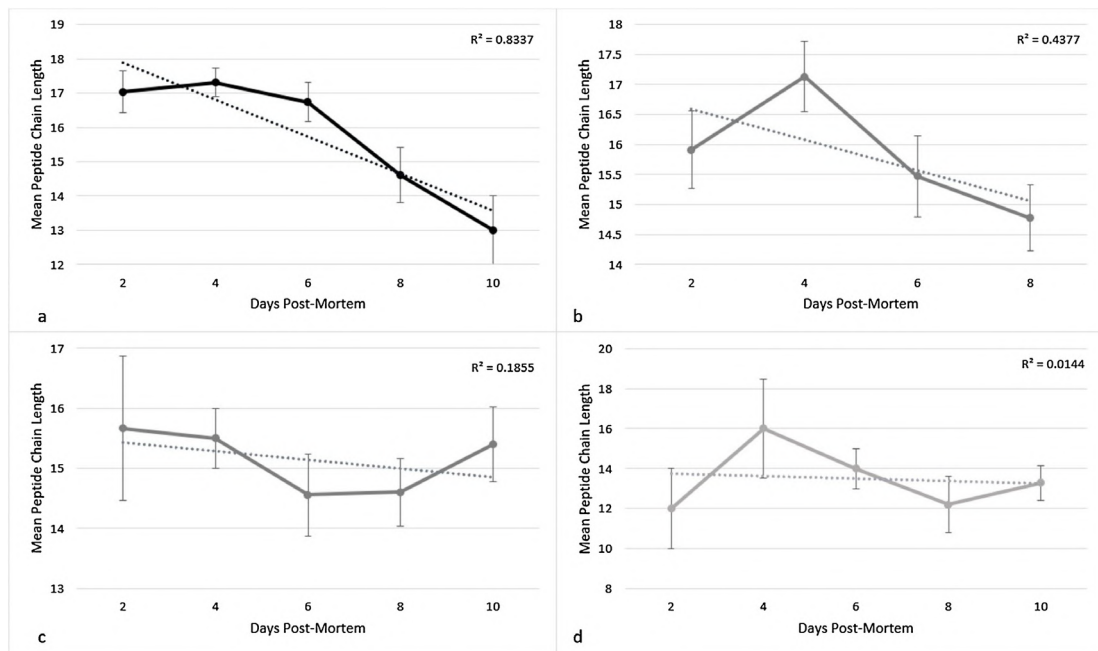


Fig. 4. Average chain length of peptides associated with (a) haemoglobin subunit alpha, (b) haemoglobin subunit beta, (c) beta-enolase, and (d) creatine kinase. Samples collected from Cadavers 1, 2, 3, 4, 5, 6, 7 and 8 on analysis days 2–10 (ADD 49–240) in summer.

assist in the estimation of time since death in both the early and later post-mortem stages.

The consistent identification of peptides derived from haemoglobin subunits alpha and beta, creatine kinase and beta-enolase

appear to be significant in this regard. As in our previous work [32], proteins and peptides were classified as being present if, when averaged on each sampling day, they were identified more than 50% of the time during the course of each trial period. A combined

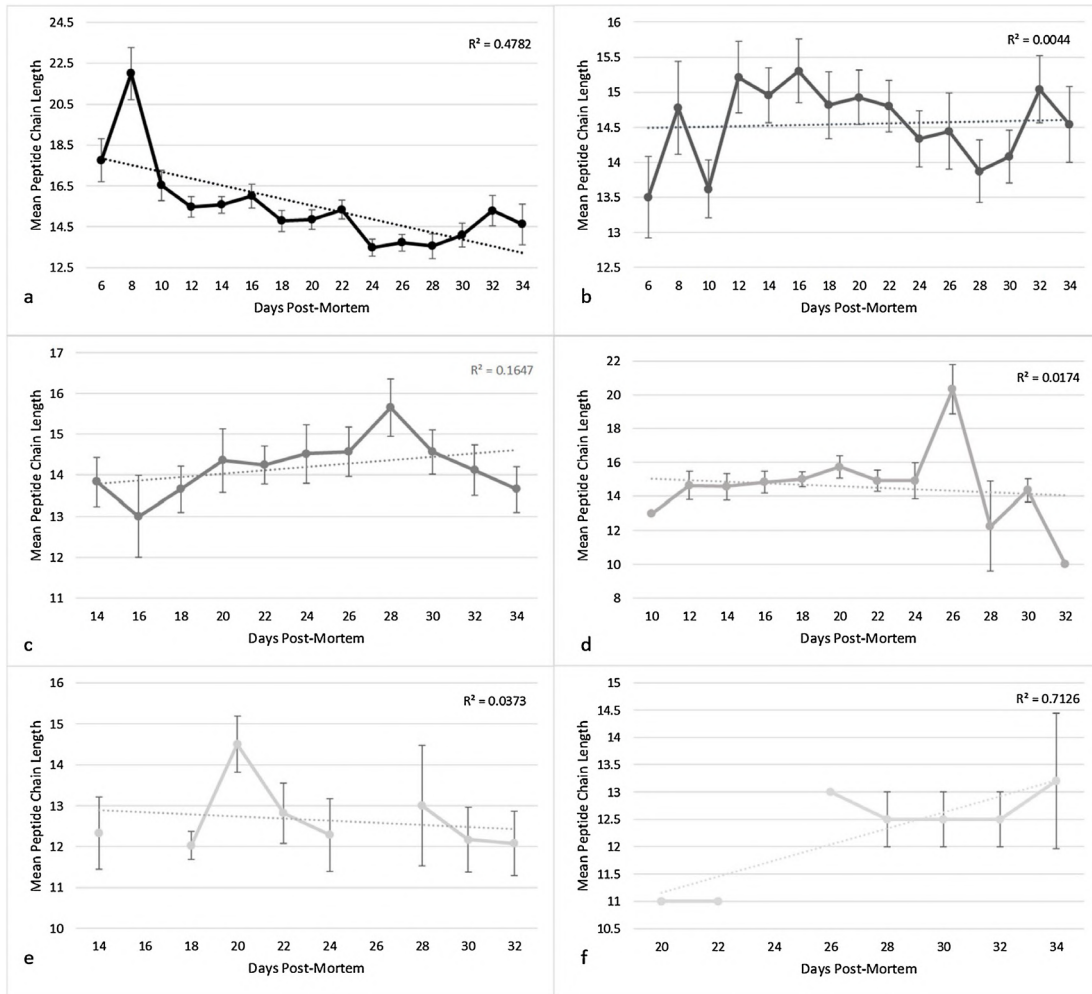


Fig. 5. Average chain length of peptides associated with (a) haemoglobin subunit alpha, (b) haemoglobin subunit beta, (c) beta-enolase, (d) creatine kinase, (e) L-lactate dehydrogenase, and (f) troponin C2. Samples collected from Cadavers 1, 2, 3, 7 and 8 on analysis days 6–34 (ADD 84–429) in winter.

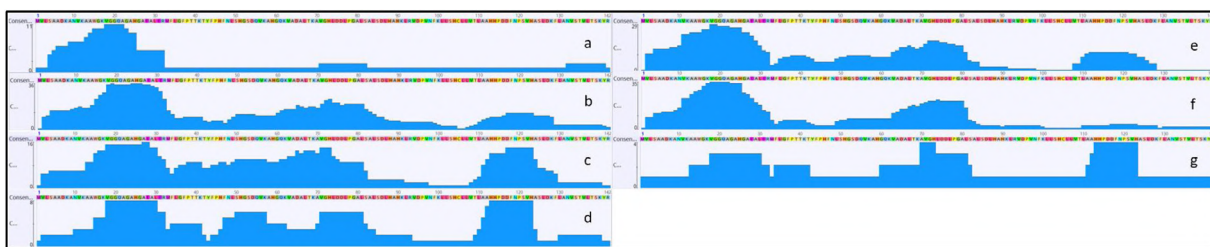


Fig. 6. Mean degradative patterns for haemoglobin subunit alpha. Peptides were detected in decomposition fluid samples collected from Cadavers 1, 2, 3, 7, and 8 on analysis days (a) 6 (ADD 84), (b) 14 (ADD 182), (c) 22 (ADD 234), and (d) 30 (ADD 385) in winter and Cadavers 1, 2, 3, 4, 5, 6, 7, and 8 on analysis days (e) 2 (ADD 49), (f) 6 (ADD 152), and (g) 10 (ADD 240) in summer.

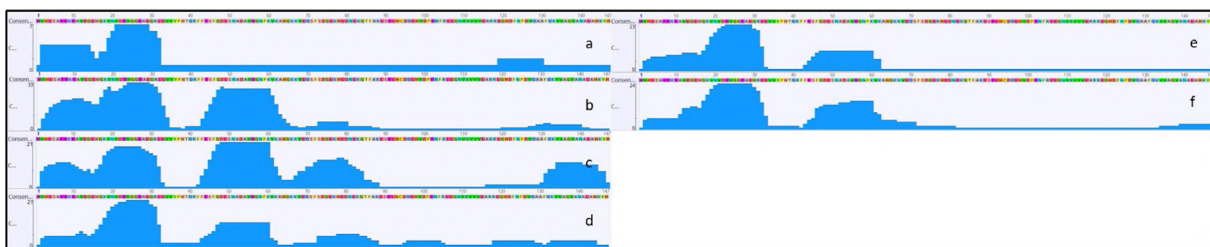


Fig. 7. Mean degradative patterns for haemoglobin subunit beta. Peptides were detected in decomposition fluid samples collected from Cadavers 1, 2, 3, 7, and 8 on analysis days (a) 6 (ADD 84), (b) 14 (ADD 182), (c) 22 (ADD 234), and (d) 30 (ADD 385) in winter and Cadavers 1, 2, 3, 4, 5, 6, 7, and 8 on analysis days (e) 2 (ADD 49) and (f) 6 (ADD 152) in summer.

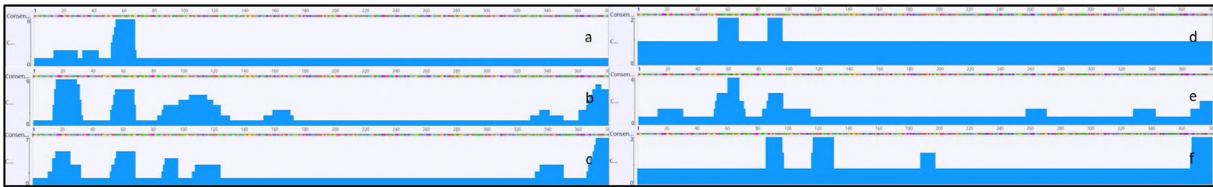


Fig. 8. Mean degradative patterns for creatine kinase. Peptides were detected in decomposition fluid samples collected from Cadavers 1, 2, 3, 7, and 8 on analysis days (a) 14 (ADD 182), (b) 22 (ADD 234), and (c) 30 (ADD 385) in winter and Cadavers 1, 2, 3, 4, 5, 6, 7, and 8 on analysis days (d) 2 (ADD 49), (e) 6 (ADD 152), and (f) 10 (ADD 240) in summer.

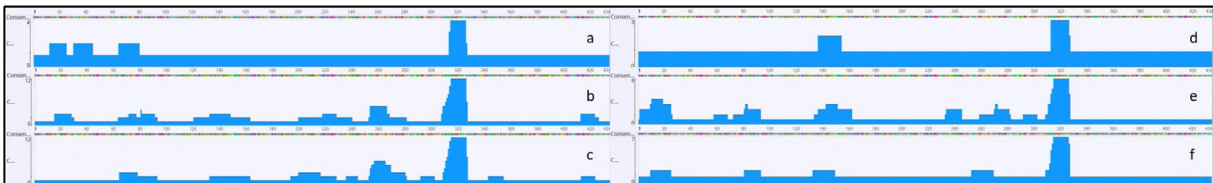


Fig. 9. Mean degradative patterns for beta-enolase. Peptides were detected in decomposition fluid samples collected from Cadavers 1, 2, 3, 7, and 8 on analysis days (a) 14 (ADD 182), (b) 22 (ADD 234), and (c) 30 (ADD 385) in winter and Cadavers 1, 2, 3, 4, 5, 6, 7, and 8 on analysis days (d) 2 (ADD 49), (e) 6 (ADD 152), and (f) 10 (ADD 240) in summer.

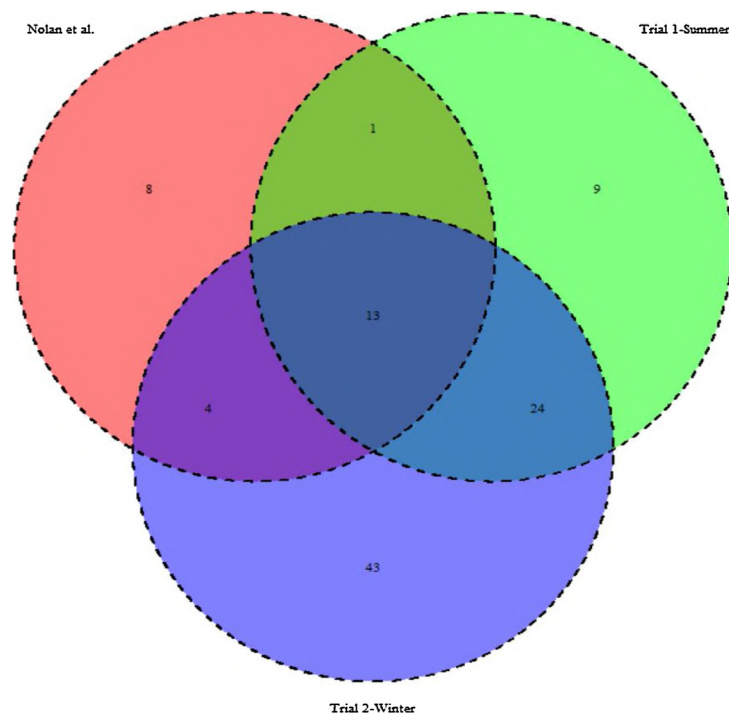


Fig. 10. Venn diagram reporting common peptides present (detected $\geq 50\%$ of the time across trial period) as recorded by Nolan et al. [32], and in this study (Summer and Winter).

total of 47 peptides were classed as being present in summer, and 95 peptides in winter. Of the 142 peptides detected in both trials, 37 were found to be common (Fig. 10).

In the current trials, 22 peptides originated from haemoglobin subunit beta, 1 from haemoglobin subunit alpha, 4 from beta-enolase, and 2 from creatine kinase (Table 1). When cross-referenced against previous findings, 13 peptides occurred consistently, regardless of trial conditions (Fig. 10). This commonality together with the identification of peptides generated from those regions of the protein most susceptible to hydrolysis,

reinforces the view that characterisation of peptides generated during decomposition is valuable in estimating time since death.

4. Conclusion

Decomposition fluid is an abundant resource after death and though it is a complex matrix, analysis of its protein and peptide content has potential for estimating the post-mortem interval. The consistent identification of several protein-specific peptides generated during decomposition trials conducted under different

temperature regimes suggests that this approach may be useful in estimating time since death for post-mortem periods of varying length. When temperature was accounted for, and when expressed in ADD, degradation patterns for the targeted proteins remained consistent between summer and winter trials. Further research is now underway to quantify the peptides identified in this study to further evaluate their usefulness in the estimation of post-mortem interval.

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CRediT authorship contribution statement

Ashley-N'Dene Nolan: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Robert J. Mead:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration. **Garth Maker:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration. **Scott Bringans:** Writing - review & editing, Supervision, Project administration, Funding acquisition. **Samuel J. Speers:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration.

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