# Mass Spectrometry Imaging to Investigate Carnitine Metabolism during Brain Ageing

**Shazia Khan**[1], Ruth Andrew[1] and Nicholas J W Rattray[2]

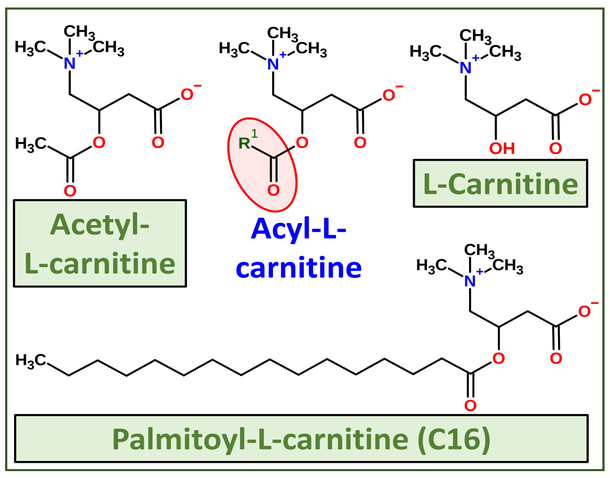
[1]Centre for Cardiovascular Science and Mass Spectrometry Core Edinburgh Clinical Research Facility, University of Edinburgh, UK

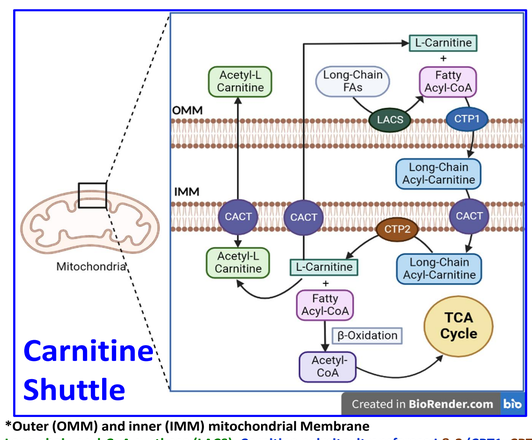
[2]Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow, UK

**Email:** ***shazia.khan@ed.ac.uk***

## Introduction

- **L-Carnitine** and **acyl-L-carnitines (ACs)** are synthesised endogenously in kidney, liver and brain and play a vital role in energy metabolism.
- L-Carnitine transports activated long-chain fatty acids (FAs) across mitochondrial membranes through a series of reactions called the "**carnitine shuttle**" for subsequent β-oxidation and energy production which prevent accumulation of long chain fatty acids and long chain acyl-CoAs within cells.
- Ageing is associated with decline in mitochondrial function and cellular energy levels whereby dysregulation carnitine metabolism is highly correlated to poor ageing phenotypes.
- **Our global aim is to understand dysregulation of carnitine metabolism during the ageing process by determine the differences in composition and distribution of L-carnitine and ACs of various FA chains within aged brains.**




*Outer (OMM) and inner (IMM) mitochondrial Membrane
Long-chain acyl-CoA synthase (LACS), Carnitine palmitoyltransferase I & 2 (CPT1; CPT2)
Carnitine/acylcarnitine translocase (CACT)

## Hypothesis

- Decline in long-chain ACs level within cells is related to disordered transportation and reflects lower cellular energy upon ageing.
- Mass Spectrometry Imaging (MSI) can be used to spatially profile and identify key differential in chemical features within brain tissue

## Aim

Develop a robust MSI protocol to image L-carnitine and acyl-L-carnitines of various chain lengths (C0-18) simultaneously in brain

## Experimental Plan

C57BL-6 male mice; n=3/group

3 months

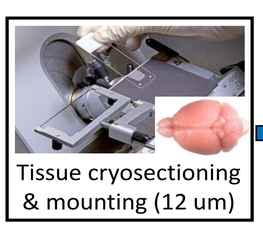
12 months

Culled

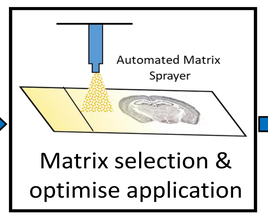
**Samples Collection**

Brain tissues were snap frozen in $LN_2$ vapour & stored -80$^0$C until analysis.

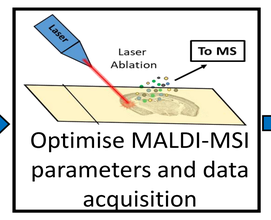
## MALDI Imaging Work Flow



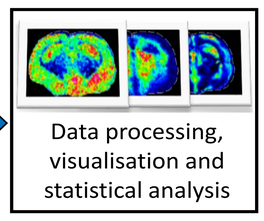
Tissue cryosectioning & mounting (12 um)




Matrix selection & optimise application




Optimise MALDI-MSI parameters and data acquisition



Data processing, visualisation and statistical analysis

## Material and Methods

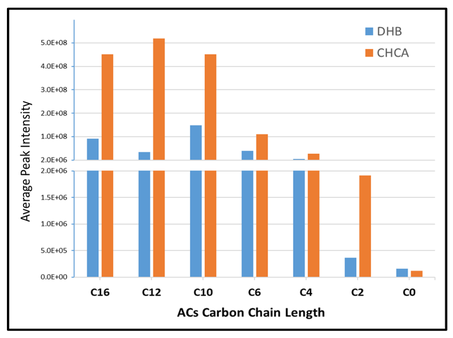
- Matrix assisted laser desorption ionisation (MALDI) MSI was performed on Waters Synapt G2-Si.
- Sample preparation and instrument conditions were optimised and validated using ACs standards of various chain lengths; L-carnitine (C0), acetyl-(C2), butyryl-(C4), hexanoyl-(C6), decanoyl-(C10), lauroyl-(C12), myristoyl-(C14), palmitoyl-(C16) and stearoyl-(C18).
- Two MALDI matrices, 2,5-dihydroxybenzoic acid (DHB; 40mg/mL in 50% MeOH + 0.2% TFA) and α-cyano-4-hydroxycinnamic acid (CHCA; 10mg/mL in 50% ACN + 0.2% TFA) were evaluated.
- Matrix mixture were sprayed using automated M3 TM sprayer (HTX technologies).

## Results and Discussion

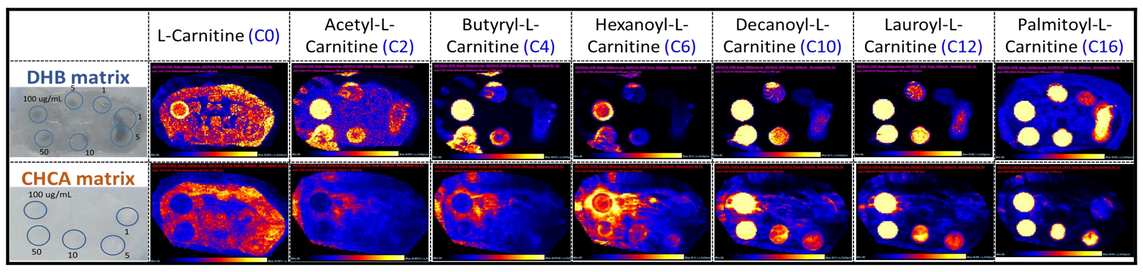
All carnitines readily ionised as $[M+H]^+$ by MALDI using CHCA and DHB matrices however ion abundances varied with FA chain length as follows; C16 ≥ C12 ≥ C10 > C6 ≥ C4 > C2 > C0.

**Selected Carnitine Standards for MSI Analysis**

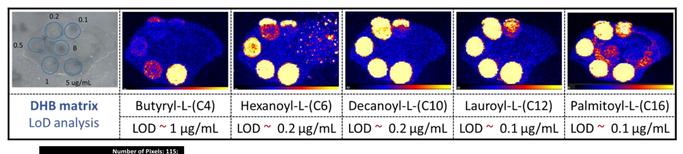
| | Carnitine ($R_1$-C=O Chain Length) | Molecular Formula | *m/z* $[M+H]^+$ |
|---|---|---|---|
| 1. | Stearoyl-L-Carnitine (C18) | $C_{25}H_{49}NO_4$ | 428.3739 |
| 2. | Palmitoyl-L-Carnitine (C16) | $C_{23}H_{45}NO_4$ | 400.3426 |
| 3. | Myristoyl-L-Carnitine (C14) | $C_{21}H_{41}NO_4$ | 372.3113 |
| 4. | Lauroyl-L-Carnitine (C12) | $C_{19}H_{37}NO_4$ | 344.2800 |
| 5. | Decanoyl-L-Carnitine (C10) | $C_{17}H_{33}NO_4$ | 316.2487 |
| 6. | Hexanoyl-L-Carnitine (C6) | $C_{13}H_{25}NO_4$ | 260.1861 |
| 7. | Butyryl-L-Carnitine (C4) | $C_{11}H_{21}NO_4$ | 232.1548 |
| 8. | Acetyl-L-Carnitine (C2) | $C_9H_{17}NO_4$ | 204.1235 |
| 9. | L-Carnitine (C0) | $C_7H_{15}NO_3$ | 162.1130 |

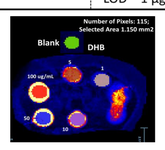



On tissue standard spots (100-1 µg/mL) and limit of detections (LoDs) analysis suggested DHB is better choice of matrix for carnitines imaging by MALDI

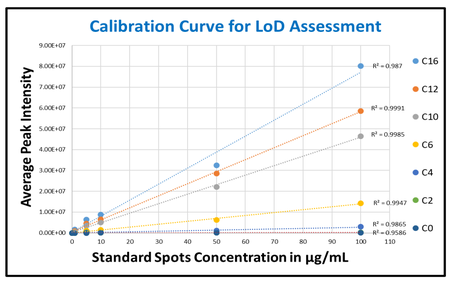



Region of interest (ROI) analysis of standards spots depicted different LoDs depends on FA chain lengths; C16 (< 0.1 µg/mL) > C12 (~0.1 µg/mL) > C10 (~0.2 µg/mL) > C6 (~0.2 µg/mL) > C4 (~1 µg/mL) > C2 (~5 µg/mL) > C0 (~100 µg/mL).

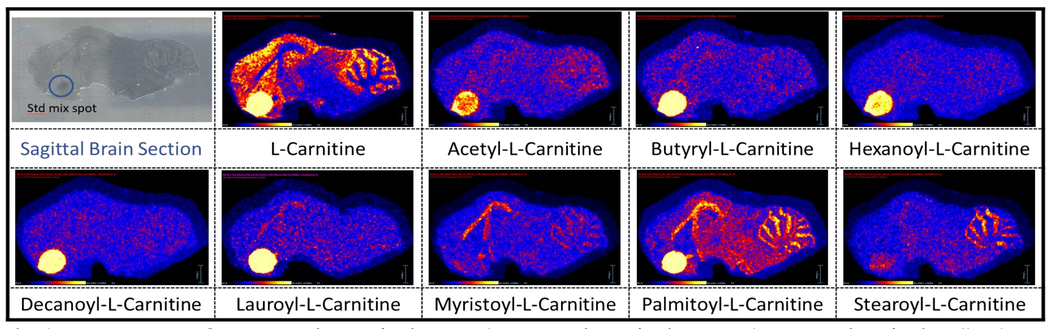



Data extracted from ROI (115 pixels; 1.150 mm$^2$ area) for LoD analysis.

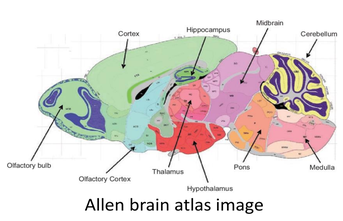



ACs MALDI-MS imaging (100 µm spatial resolution) in control mouse brain tissue showing endogenous L-carnitine highly abundant in grey-matter, in contrast to long-chain ACs (C14-18) which were present in white-matter.

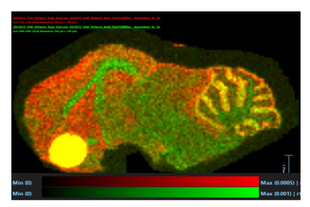


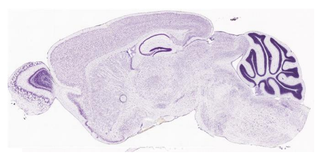

*Std mix spot contain [L-Carnitine (100ug/mL) + Acetyl Carnitine (10ug/mL) + Butyryl Carnitine (1ug/mL) + All other long chain ACs (hexanoyl + decanoyl + lauroyl + palmitoyl; 0.5ug/mL)] according to LoD for validation & identification.

Overlay images of L-carnitine (C0) & palmitoyl-L-carnitine (C16) shows C0 is more abundant in cortex, hippocampus and cerebellum grey matter, however C16 is in mid brain, thalamus, hypothalamus and cerebellum white matter.




Allen brain atlas image





## Conclusion & Future Work

- ✓ Detection and specific localisation of L-carnitine and acyl-carnitines on mouse brain tissue is possible by MALDI-MSI in positive ionisation mode.
- ✓ DHB is best choice of matrix for MALDI imaging of acyl-carnitines (C0-18).
- ❑ Next steps will involve determining the differences in composition and distribution of L-carnitine and acyl-carnitines of various chain lengths within aged mouse brains.

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