Proceedings of the Nutrition Society (2011), **70 (OCE3)**, E76 doi:10.1017/S0029665111001169

Joint Irish Section and American Society for Nutrition Meeting, 15–17 June 2011, 70th anniversary: 'Vitamins in early development and healthy ageing: impact on infectious and chronic disease'

A validation study to compare the effect of different processing protocols on measured N^{ε}-(carboxymethyl)lysine and N^{ε}-(carboxyethyl)lysine in blood

G. L. J. Hull[1], G. J. Cuskelly[1] and J. V. Woodside[2]

[1]*Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University Belfast, BT9 5AG and* [2]*School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast BT12 6BJ*

Epidemiological studies have shown that elevated plasma advanced glycation endproducts levels are associated with disease conditions such as diabetes, kidney disease and heart disease[1], thus AGE have been subsequently used as a marker for the progression of disease states[2]. CML is the best characterised AGE. Some investigators have previously employed a protocol to stabilise CML at the time of blood collection. However, it has not been shown whether this additional protocol prevents artefactual CML and/or CEL formation, and including additional steps in handling of blood samples at collection inevitably increases the costs associated with CML analysis, and reduces the likelihood of such samples being collected in large population studies. The aim of this validation study was to compare measured CML and CEL in blood samples collected under various processing conditions.

The investigation examined the effect on the level of CML and CEL in pooled samples (n 10) of (i) different blood collection tubes (clotted (serum), EDTA and lithium heparin (both plasma)), (ii) the addition of a stabilising cocktail solution (0.2 mM indomethacin, 5 mM butylated hydroxytoulene and 10 mM diethylenetriaminepentaacetic acid), (iii) use of fresh and stored (1 d and 1 week, at 4°C) stability cocktail, (iv) storing samples at 4 and 21°C for 1, 2, 4, 8 and 24 h, prior to blood centrifugation and/or addition of stabilising cocktail solution, and (v) effect of one and two freeze thaw cycles. All samples were reduced, precipitated, hydrolysed and underwent solid-phase extraction using a C_{18} cartridge. Extracts were analysed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) with the use of isotopically labelled internal standards and by reference to an external standard calibration curve[3]. Samples were extracted in triplicate (n 3). The inter-sample variability was <7%, and the intra and inter assay coefficient of variability were 5.1 and 5.7%, respectively.

The table shows the effect of time before centrifugation on CML and CEL in EDTA plasma samples, stored at room temperature.

Hour(s) before centrifugation	CML (mmol/mol lysine)	SD	CV	CEL (mmol/mol lysine)	SD	CV
0	0.12	0.01	4.23	0.05	0.004	6.82
4	0.14	0.00	3.14	0.06	0.004	6.85
24	0.12	0.01	5.26	0.07	0.004	6.66

There was no significant effect of any of the different experimental conditions tested (ANOVA). Additional processing of blood at collection does not enhance measured CML and CEL. This indicates that specific collection protocols are not required for measuring CML and CEL, thus indicating that many samples currently stored for epidemiological studies could be used for CML and CEL analyses.

1. Semba RD, Nicklett EJ & Ferrucci L (2010) *J Gerontol A Biol Sci Med Sci* **65**, 963–975.
2. Vlassara H & Palace MR (2002) *J Intern Med* **251**, 87–101.
3. Teerlink T, Barto R, Ten Brink HJ *et al.* (2004) *Clin Chem* **50**, 1222–1228.