Institution: University of East Anglia

Unit of Assessment: 1 – Clinical Medicine

Title of case study:

Accurate measurement of Vitamin D to develop guidelines for health

1. Summary of the impact:

Use of our new Tandem Mass Spectrometry (MS) technology for measuring 25 Hydroxy Vitamin D (25OHD) has had both major clinical and economic impacts on:

- patient care via the National Osteoporosis Society Guideline (April 2013) '*Vitamin D and Bone Health - A Practical Clinical Guideline for Patient Management*'
- accredited laboratory assay methodologies where reports from the Vitamin D External Quality Assessment Scheme show increasing use of Tandem MS in recognition of the need to accurately measure both $25OHD_2$ and D_3
- army recruits through the amended Ministry of Defence training policy which now incorporates an approach to injury/stress fracture prevention and improvements in Vitamin D status
- the NHS through uptake of the Tandem MS 25OHD assay.

2. Underpinning research

Before our development of Tandem Mass Spectrometry, Vitamin D was measured by a variety of immunoassays with poor standardisation that could result in over or under estimation of Vitamin D levels leading to erroneous diagnosis and incorrect treatment. This case study stems from a body of research into the measurement of $25OHD_2$ and D_3 using a High Performance Liquid Chromatography (HPLC) tandem Mass Spectrometry (MS) methodology developed by Fraser and Dutton. This work commenced in Liverpool leading to an early method for Tandem MS analysis (see for example: *Dutton, J and* ***Fraser, WD*** *The Technical and Clinical benefits from Measuring 25 OH Vitamin D by LC-MS/MS. Mass Matters* ***2010*** *62:13-15*) and continued with significant scientific and technical developments to sample preparation and assay technology following Fraser's move from Liverpool to UEA in April 2011.

The current technology allows measurement of both serum $25OHD_2$ and D_3 with precision, accuracy and sensitivity, with sufficient throughput to enable large-scale studies to be performed with confidence. The major clinical advantage of the method is the ability to estimate accurately both $25OHD_2$ and D_3. All immunoassays have poor Ab cross reactivity with D_2 resulting in underestimation of $25OHD_2$ and, depending on $250HD_3$ standardisation, total 250HD status. The specific detection of $25OHD_2$ also allows the detection of toxicity (hypercalcaemia) due to excessive levels of $25OHD_2$ and hence the avoidance of unnecessary and expensive patient investigation for malignancy as a cause of hypercalcaemia.

The introduction at UEA of high-throughput extraction technology combined with the excellent sensitivity of Tandem MS has allowed large numbers of samples to be measured for several studies. This work has been underpinned by grant income of £3.5M since 2005. The data obtained are unique as the biochemical techniques developed allow measurement of Vitamin D metabolites with a sensitivity, precision and accuracy not available to the majority of researchers. This new technique has now superseded immunoassays in many instances and has been used in a number of clinical trials overturning the previously held consensus on Vitamin D therapy (1).

The studies performed at UEA to date, and where $25OHD_2$ has significant association with, or effect on, disease, are extensive and include prostate cancer, cardiovascular disease, respiratory disease (COPD), diabetes, depression, dermatological conditions, problems of pregnancy including eclampsia, non-clinical psychotic experiences, behavioural problems, academic performance, cortical bone development and increased stress fracture incidence. A notable recent clinical result arising from the research at UEA is that there is no association between maternal

Vitamin D status in pregnancy and offspring bone-mineral content in late childhood (2).

During 2011-2013, research at UEA established that $25OHD_2$ supplementation at currently recommended doses is not effective in changing several clinical outcomes. This is having major scientific, social and economic effects, resulting in new approaches to investigation of Vitamin D effects and altering data interpretation. This research has been quoted extensively and incorporated into several meta-analyses of the role of $25OHD_2$ in disease, and in attempts to define the optimal therapeutic thresholds for circulating $25OHD_2$ (3).

Associations of low circulating $25OHD_2$ with poorer outcomes in disease has led to the funding of many important prospective randomised studies investigating Vitamin D supplementation in treatment and prevention of disease.

UEA researchers:
William Fraser: UEA Professor of Medicine since April 2011 and Principal Investigator of the UEA research team.
Jonathan Tang: UEA Bio-analytical Facility Manager since April 2011.

There has been close collaboration in the clinical studies with investigators from Bristol and Aberdeen.

3. References to the research

(UEA authors in bold)

1) **Fraser WD**, Milan AM
Vitamin D assays: past and present debates, difficulties, and developments
Calcif Tissue Int. **2013** 92:118-27
doi: 10.1007/s00223-012-9693-3
Significance: Quoted in the National Osteoporosis Society Practical Guideline on Vitamin D for UK physicians. The basis for the analytical recommendations in the guideline.

2) Lawlor DA, Wills AK, Fraser A, Sayers A, **Fraser WD**, Tobias JH
Association of maternal Vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study
Lancet **2013** 381:2176-83
doi: 10.1016/S0140-6736(12)62203-X
Significance: Editorial Comment on Vitamin D supplementation of pregnant women in the UK.

3) MacDonald HM, Wood AD, **Tang JC**, **Fraser WD**
Comparison of Vitamin D_2 and Vitamin D_3 supplementation in increasing serum 25-hydroxyvitamin D status: a systematic review and meta-analysis
American Journal of Clinical Nutrition **2012** 96:1152-3 (author reply 1153-4)
doi: 10.3945/ajcn.112.046110
Significance: Discussion of the importance of using Vitamin D_3 supplementation in preference to Vitamin D_2 in deficient patients.

Key grants supporting this research:

MRC Avon Longitudinal Study of Parents and Children (ALSPAC) (2008-12) £1,280,874
Lawlor DA, Davey Smith G, Evans DMF, **Fraser WD**, Guthrie P, Hypponen ET

MRC (2008-13) £663,429
Fraser WD, Selby P, Langston A, Ralston S

Arthritis Research UK (2009-14) £483,127
Ralston S, Langston A, Campbell M, **Fraser WD**

Arthritis Research UK (2010-13) £211,899
Hauser B, Riches P, **Fraser WD**, Ralston S

MRC Population and Systems Medicine Board (2011-14) £489,444
Parekh D, Dancer RC, Lax S, Cooper MS, Martineau AR, **Fraser WD**, Tucker O, Alderson D, Perkins GD, Gao-Smith F, Thickett DR

4. Details of the impact

Our new Tandem MS technology for simultaneous measurement of both $25OHD_2$ and $25OHD_3$ has had both clinical and economic impacts.

Impact on Patient Care: For the wider population the common Vitamin D supplements provide Vitamin D_2 whereas the common (immunoassay) techniques for measuring Vitamin D levels are unable to recognise this form, frequently resulting in significant underestimation of total Vitamin D levels. The ability to detect both $25OHD_2$ and $25OHD_3$ simultaneously therefore has two major patient benefits: the avoidance of over-supplementation - with the associated risk of toxicity; and the unnecessary investigation of possible causes of non-parathyroid related hypercalcaemia.

The 2013 National Osteoporosis Society guidelines '**Vitamin D and Bone Health. A Practical Clinical Guideline For Patient Management**' recommend that 25OHD should be measured by a method able to clearly distinguish $25OHD_2$ and D_3. Authorities involved in Vitamin D research now recognise the problems arising from the lack of specificity inherent in immunoassays and therefore recommend the use of Tandem MS technology. (corroborating source A)

Impact on National Quality Assessment Methodologies: The importance of the Tandem MS measurement technology has been recognised by laboratories accredited to analyse samples for Vitamin D resulting in significant expansion of use of this technology in NHS laboratories and research establishments. This is clearly apparent from the regular reports of the Vitamin D External Quality Assessment Scheme (DEQAS). The DEQAS reports over a 10 year period highlight the increasing use of Tandem MS technology by participants. For example, there were no users of Tandem MS technology in 2004 and, following our development of the new assay, Tandem MS usage rose to 12.8% of users in 2013. (corroborating source B)

Impact on Army Recruits via MOD Training Programmes: Based on the underpinning research reported in references 1, 2 and 3 in section 3, Fraser wrote confidential reports for the MOD which led to three collaborative programmes of work between the Army Recruitment and Training Directorate and UEA. As a result, the MOD recognised that a high percentage of recruits have significant Vitamin D deficiency and that female recruits have a particular problem leading to high bone turnover and predisposition to injury, especially post-partum.

This has resulted in:

- a review of the nutritional recommendations for all recruits in training
- a revised medical policy to protect postpartum service personnel in a medically downgraded capacity for 12 months postpartum
- an alteration in the training programme for female recruits – who are now trained separately to male recruits.

(corroborating source C)

These changes were based on the following findings from the collaborative work:

a) Vitamin D and bone health: We have confirmed an association between Vitamin D deficiency and impaired bone health in female Army recruits, and a high incidence of Vitamin D deficiency in both men and women entering / exiting training.

b) Pregnancy and postpartum: The postpartum period is recognised to be a vulnerable time for female military personnel on return to physically demanding roles with an increased risk of musculoskeletal injury. Our work has shown increased bone turnover in a representative population of women over a 6-month period.

c) Gender differences in bone density and morphology: It is generally known that women are more likely to sustain a stress fracture injury during training than men. Our collaborative work has shown that this is due to differences in bone morphology between male and female recruits. These findings have also provided evidence for individual consideration of female-to-male transgender cases.

Impact on the NHS Economy: The Tandem MS technology has had significant economic impact on the NHS. For example, the Norfolk and Norwich University Hospital is an early adopter of this new assay where it is now the routine method for measurement of 25OHD. The cost per sample for a Tandem MS analysis is currently £12.50 compared to the cost of analysis by immunoassay (the previous method of choice) which is £18.50. The NNUH 'hub' currently requests analysis of 3800 samples per annum resulting in a cost saving of £125,000 in this region alone. (corroborating source D)

5. Sources to corroborate the impact

A. **Vitamin D and Bone Health. A Practical Clinical Guideline For Patient Management (2013)** National Osteoporosis Society
http://www.nos.org.uk/document.doc?id=1352

The guideline is endorsed by the Bone Research Society, British Geriatrics Society, British Orthopaedic Association, International Osteoporosis Foundation, Primary Care Rheumatology Society, Royal College of Nursing, Royal Pharmaceutical Society, Society for Endocrinology, British Dietetic Association, UK Clinical Pharmacy Association and the Paget's Association.

Specific references to UEA work are on pages 9, 10, 22, 24.

The guideline states (page 11):

Notwithstanding the various technical aspects of measuring Vitamin D, there are a few simple considerations that need to be applied from a clinical perspective:

- *Measurement of serum 25OHD is the best way of estimating Vitamin D status.*
- *The assay used should have the ability to recognise all forms of 25OHD (D2 or D3) equally. In practice, this means that it should use either HPLC or, more likely, tandem MS. None of the immunoassays offer the ability to recognise all forms of 25OHD.*

(copy held on file at UEA)

B. **Vitamin D External Quality Assessment Service (DEQAS)** comparison data between 2004 and 2013:

Date	Sample	N	LC-MS	LC-MS percentage
Apr-2004	251	97	0	0.00%
Apr-2005	271	141	2	1.42%
Apr-2006	291	161	9	5.59%
Apr-2007	311	229	20	8.73%
Apr-2008	331	339	35	10.32%
Apr-2009	351	565	53	9.38%
Apr-2010	371	774	86	11.11%
Apr-2011	391	986	104	10.55%
Apr-2012	411	1116	133	11.92%
Apr-2013	431	1102	141	12.79%

(data held on file at UEA)

C. Letter on behalf of the **Ministry of Defence / Army Recruitment and Training Directorate**, which details the recommendations on army training and dietary intake of Vitamin D for all new recruits.

(held on file at UEA)

D. **NHS Vitamin D analysis sample figures**: data provided by the Norfolk and Norwich University Hospital and held on file at UEA.