Final grant report form: Norman Hayward Fund

PROJECT/STUDY TITLE:	Markers of equine laminitis predisposition: searching for a potential future diagnostic test
PRINCIPAL APPLICANT(S)	Nicola Menzies-Gow
GRANT AWARDED (DATE):	January 2014

Lay summary of project outcomes, achievements and potential impact:

Twelve potential diagnostic markers of future laminitis development were measured in blood samples which had been collected from 446 ponies. These animals had no history of previous laminitis and were followed for three years to determine which developed laminitis in the future. Assays had first to be validated for five of the markers. Circulating concentrations of the fat-derived hormone adiponectin and of the hormone insulin before (baseline) and 19 hours after injection of the corticosteroid dexamethasone were significantly associated with laminitis development in the following one, two and three years. The accuracy of adiponectin to separate animals which did, from animals which did not, develop laminitis was fair and a cut of value of 2.50□g/ml gave acceptable sensitivity (the proportion of positives that are correctly identified as such) and specificity (the proportion of negatives that are correctly identified as such) values. The accuracy of baseline insulin concentration to similarly separate animals was good (two years) to fair (one and three years) and a cut of value of 21.8□iu/ml gave acceptable sensitivity and specificity values. The accuracy of insulin concentration after dexamethasone administration to separate animals was poor and a cut of value of 105.6□iu/ml gave fair sensitivity and specificity values. Combinations of these biomarkers did not improve their predictive value. Further prospective cohort studies that examine animals more frequently enabling these three potential biomarkers to be measured within a shorter time frame in relation to the onset of laminitis or that include a much larger number of animals are warranted to assess their usefulness further. If they prove useful when applied to the horse population, their measurement may allow management of the at-risk animals to be changed such that the frequency of episodes of laminitis is significantly reduced or completely prevented, thus significantly improving their welfare.

Detailed progress against original objectives: List outcomes against original objectives. Discuss what has been achieved, including any statistical analysis completed as part of the project.

The original aim of the project was to discover potential diagnostic markers for laminitis predisposition before an animal has had the disease or when its previous history is unknown. The markers selected for evaluation in this proposed study were based on the hypothesis that susceptibility to pasture-associated laminitis is associated with a metabolic phenotype including obesity and insulin dysregulation (ID), similar to that seen in human metabolic syndrome (HMS). Thus, the same pathologic mechanisms that underlie the cardiovascular disease associated with HMS, including changes in insulin signaling, inflammatory cytokines and endothelial dysfunction, could contribute to laminitis. We hypothesised that circulating concentrations of markers of ID, inflammation and endothelial dysfunction would be significantly higher in those animals that subsequently developed laminitis compared to those that did not.

Blood samples, from a cohort of 446 ponies with no previous history of laminitis, had been previously collected. Plasma concentrations of adiponectin and leptin were measured using radioimmunoassays, and plasma von Willebrands factor (vWF) and C-reactive protein concentrations were measured using ELISAs; all of these methods had been previously validated for use in the horse. Plasma triglyceride concentrations were measured by a commercial laboratory. Serum insulin-like growth factor (IGF)-1, IGF binding protein (IGFBP)-1, IGFBP-3, plasma P-selectin and serum E-selectin were measured using human and equine ELISAs; these assays required prior validation including assessment of intra and inter-assay repeatability, spiked recovery and dilutional parallelism in order to ensure that they performed satisfactorily.

The ponies were monitored for three years following sample collection and after one, two and three years 18 (4%), 30 (7%) and 44/446 (10%) animals had developed laminitis; 416 (93%), 374 (84%) and 348 (78%) remained non laminitic; and twelve (3%), 42 (9%) and 54 (12%) had been euthanased for reasons other than laminitis, respectively. These values represent cumulative figures over the three-year period and none of the animals were lost to follow-up.

Univariable logistic regression was first used to assess risk factors associated with the outcome (namely laminitic after one year or not; laminitic after two years or not; laminitic after three years or not) individually. Those risk factors with $p<0.1$ were entered into a multivariable logistic regression and any risk factors with $p>0.05$ sequentially removed until all the risk factors had $p\leq0.05$ in the final model. ROC curves were constructed for those risk factors remaining in the final model. The accuracy of the test to separate animals into those which did or did not subsequently develop laminitis was determined by calculating the area under the ROC curve (AUC) whereby an area of 0.90-1 is excellent, 0.80-0.90 good, 0.70-0.80 fair, 0.60-0.70 poor and 0.50-.60 fail. In addition, the co-ordinates of the ROC curve were then used to determine the cut off value which maximised the specificity and sensitivity and the corresponding positive (PPV) and negative predictive values (NPV) using these cut-off values were calculated.

Plasma adiponectin and serum insulin pre (baseline) and post dexamethasone concentrations remained within the three multivariable logistic regression models and were significantly ($p<0.05$) associated with laminitis development after one, two and three years. The accuracy of plasma adiponectin concentration to separate animals which did, from animals which did not, develop laminitis was fair (one, two and three years) and a cut of value of 2.50□g/ml gave acceptable sensitivity (78%) and specificity (79%) values. The accuracy of baseline insulin concentration to similarly separate animals was good (two years) to fair (one and three years) and a cut of value of 21.8□iu/ml gave acceptable sensitivity (78%) and specificity (67%) values. The accuracy of insulin concentration after dexamethasone administration to separate animals was poor and a cut of value of 105.6□iu/ml gave fair sensitivity (69%) and specificity (68%) values for the development of laminitis in the next twelve months. Combinations of these biomarkers did not improve their predictive value.

Low plasma IGF-1 concentration was also a risk factor, but only at one time point (two years). In contrast to previous studies, the development of laminitis was not associated with hyperleptinemia or hypertriglyceridemia. In addition, there was no association with circulating CRP, IGFBP-1, IGFBP-3, sE-selectin, p-selectin or vWF antigen concentrations.

Thus, the main outcomes of this project are:

1) The demonstration that low plasma adiponectin as well as high basal and post dexamethasone serum insulin concentrations are consistent risk factors for the future development of laminitis in animals prior to disease occurrence.

2) The generation of possible cut-off values (when using the same assays as this project) for these potential biomarkers of future laminitis development.

Further prospective cohort studies that examine animals more frequently, thereby enabling these biomarkers to be measured within a shorter time frame in relation to the onset of laminitis, or that include a much larger number of animals are warranted to assess these risk factors further. If they prove useful, when applied to the horse population their measurement may significantly reduce the frequency of, or even completely prevent episodes of laminitis in predisposed animals through targeted implementation of preventative measures.

Were there any challenges or barriers/modifications to the project? Explain the nature of and reasons for any changes in project focus, scope, delivery, schedule or evaluation.

The only obstacles to completion of the project were the validation of ELISAs for measurement of serum IGFBP-1, IGFBP-3, and sE-selectin and plasma P-selectin concentrations. There were no assays that have been reported to have been previously validated in horses. ELISAs were satisfactorily validated for all these biomarkers.

Provide details of knowledge transfer activities to date and any future plans/actions.
The preliminary findings were presented to equine veterinarians at European College of Equine Internal Medicine Annual Congress in November 2015 and published in the conference proceedings. The abstract will also be published in Equine Veterinary Journal in due course. The results have been submitted for consideration for publication in Equine Veterinary Journal and have been through first review where the referees have raised a number of questions which we are currently addressing.
Provide details of any original peer-reviewed research papers, book chapters and books/monographs that have resulted directly from your work supported by this grant.
The final results have been submitted for consideration for publication in Equine Veterinary Journal.
Have any other funding bodies been involved in supporting the development of the work supported by this grant?
The samples analysed using the support from this grant were generated as part of a prospective cohort study funded by Pet Plan Charitable Trust, WALTHAM Centre for Pet Nutrition and the Laminitis Trust.
Have the results been published? If yes please state when:
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Use the space below for any other relevant information you wish to report on.
An application has been made to the Norman Hayward Fund for a project grant which is the next logical extension of the most important result obtained from this project, namely further investigating the role of hypoadiponectinemia in laminitis predisposition.