Preface

This issue contains 20 papers devoted to the problem of laminitis, a condition that causes very considerable distress both to affected horses and to their owners. Further, their condition is largely one affecting smaller breeds kept largely for pleasure riding. Thoroughbreds and performance horses tend to suffer secondary to surgical or medical misfortunes. The papers contained in this issue are from research workers dedicated to establishing the means of prevention of this painful condition, and full publication of their work is therefore essential to progress. Those who have given financial support for the publication of this issue, namely Dodson & Horrell, RIRDC and BEVA Trust, have provided a valuable stepping stone in the aim towards improving the welfare of horses.

I am very grateful to the Guest Editors, Professor Leo Jeffcott and Dr Celia Marr, for the substantial amount of work that they have contributed to this Laminitis Special Issue.

P. D. ROSSDALE
EVJ Editorial Office

Editorials

Cushing’s syndromes, insulin resistance and endocrinopathic laminitis

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>Advanced glycation endproducts</td>
</tr>
<tr>
<td>BW</td>
<td>Black walnut</td>
</tr>
<tr>
<td>ECD</td>
<td>Equine Cushing’s disease</td>
</tr>
<tr>
<td>GC</td>
<td>Glucocorticoid</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>NEFA</td>
<td>Nonesterified fatty acid</td>
</tr>
<tr>
<td>PPID</td>
<td>Pituitary pars intermedia dysfunction</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
</tbody>
</table>

This issue contains two articles which throw light on the relationship of biochemical indices and serum insulin concentrations in equine Cushing’s disease (Keen et al. 2004; McGowan et al. 2004). These add to the substantial body of work published during the last decade that serves to improve our understanding of laminitis. Most of this research has focused on laminitis associated with gastrointestinal disease, Gram-negative endotoxaemia and black walnut (BW) toxicosis. Laminitis is commonly recognised in association with pituitary pars intermedia dysfunction (PPID, equine Cushing’s disease) and as a potential risk of glucocorticoid (GC) treatments, but significantly fewer publications have addressed the association between laminitis and pathological conditions of the horse’s endocrinological system (Schott 2002). This difference in research emphasis can be partly explained by the availability of useful and predictable models for the purposes of studying experimentally-induced laminitis that involve administration of either starch or a soluble extract of BW via nasogastric tube (Adair et al. 2000). In contrast, the exogenous administration of GCs to horses rarely and unpredictably results in laminitis (Johnson et al. 2002). Paradoxically, using starch/BW models, results of recent research show clearly that activation of inflammatory mediators occurs during the early stages of laminitis, suggesting that GCs (anti-inflammatory agents) should theoretically protect against the development of laminitis (Fontaine et al. 2001). Therefore, an alternative explanation for development of laminitis in the face of increased GC action should be sought.

Although a satisfactory explanation for the association between GCs and laminitis is lacking, several hypothetical explanations have been suggested (Johnson et al. 2002). Specifically, GCs might act directly to cause laminitis by: affecting vascular smooth muscle in such a manner as to enhance vasospasticity and reduce blood flow; weakening
the lamellar attachment interface through dermo-epidermal atrophy or compromising keratinocyte attachment to underlying basement membrane; increasing intestinal permeability to bacterial products. Alternatively, laminitis might arise as a consequence of GC-induced insulin resistance (IR). There is increasing recognition that IR is common in horses (Johnson 2002). Moreover, the fact that pony breeds tend to be affected with IR and are at greater risk for development of laminitis compared to horses supports the theory that IR is a risk factor for laminitis (Freestone et al. 1992; Alford et al. 2001).

Insulin resistance implies that the effectiveness of insulin signalling at insulin-sensitive target cells is impaired. Important and well-recognised insulin targets include skeletal muscle and adipose tissue in which insulin normally stimulates glucose and nonesterified fatty acid (NEFA) uptake and hepatocytes in which insulin inhibits glucogenesis. Another insulin function that is impaired in IR is, by virtue of a direct action of insulin on endothelial nitric oxide production, vasodilatation (Corry and Tuck 2001). Insulin, released in response to feeding, also acts normally in the hypothalamus to suppress appetite and to signal satiety.

Factors that interfere with the action of insulin lead to glucose intolerance (relatively elevated plasma glucose concentration) and, therefore, heightened stimulation of pancreatic beta cells. Insulin resistance is characterised by hyperinsulinaemia and hyperglycaemia. In affected horses, the magnitude of resulting hyperglycaemia is typically mild because an increase in the secretion of insulin appears to compensate for reduced insulin action. A pivotal consequence of IR is the exposure of cells that are not dependent on insulin to higher-than-normal glucose delivery (‘glucotoxicity’) (Poitout and Robertson 2002). Interestingly, pathologically significant changes within these noninsulin dependent cells occur as a result of only mild elevations in glucose. Although the pathophysiological consequences of IR affect numerous different cell types in the body, the glucotoxic changes in endothelial cells have been well characterised and represent an important mechanistic link between IR and cardiovascular conditions, such as hypertension and atherosclerosis (Cosentino and Luscher 1998). Glucotoxic changes within endothelial cells have been well characterised in other species and the reader is directed elsewhere to reviews of this complex topic (Nicollerat 2000). Briefly, higher-than-normal glucose in endothelial cells leads to glycation (glycosylation) of normal cellular proteins and the production of ‘advanced glycation endproducts’ (AGEs). Resulting AGEs lead directly to lipid peroxidation within the cell and the genesis of reactive oxygen species (ROS), such as superoxide radicals (oxidative stress). Multiple endothelial cell changes occur as a result of increased ROS and have been listed elsewhere (Hayden and Tyagi 2003; Valgimigli et al. 2003). Oxidative stress-induced endothelial changes that are particularly relevant to IR-associated cardiovascular dysfunction include reduced nitric oxide production, increased endothelin production, and the adoption of a relatively pro-coagulative phenotype. Significant consequences of these changes include increased vasospasticity, hypercoagulability, and, potentially, interference with microvascular perfusion. The extent to which these changes could increase the risk for laminitis in IR-affected horses is deserving of further investigation.

Although recent studies (Johnson et al. 1998) have demonstrated an important role for extracellular matrix metalloproteinases (MMPs) in laminitis, little is known about the effects of IR on MMP regulation. Both MMP-2 and MMP-9 are activated by ROS and their expression appears to be regulated by oxidant stress (Uemura et al. 2001). It has been shown recently that MMP-9 production by vascular endothelial cells is increased by high glucose conditions and that this glucotoxic effect can be reversed with antioxidants (Ting et al. 1996). This mechanism of redox-sensitive MMP-9 expression resulting from IR provides a mechanistic explanation for the elevated MMP activity that we have observed in hoof lamellar tissues obtained from horses affected with ECD and suggests that the efficacy of antioxidants should be investigated for the treatment of laminitis associated with ECD and IR. The extent to which glucotoxic endothelial dysfunction could contribute to risk for laminitis in IR-affected horses deserves more attention. In fact, the basic mechanism pertaining to how all of these changes act together and in what sequence to cause clinically significant cardiovascular changes in man or horses has not been resolved.

Mature horses that develop obesity are clearly at risk for laminitis. In the past, these horses have been diagnosed erroneously as and treated for hypothyroidism. Experimental induction of hypothyroidism (thyroidectomy) does not lead to obesity or laminitis in horses and appropriate testing for thyroid disease (thyroid stimulation tests) yields normal results in these obese horses (circulating levels of T3 and T4 are appropriately increased following administration of either TSH or TRH). Therefore, it has been postulated that the risk for laminitis in obese horses is more appropriately associated with the development of IR. The ‘syndrome’ of obesity, IR and laminitis in mature horses has been referred to as either ‘peripheral Cushing’s syndrome’ or an equine ‘metabolic syndrome’ (Johnson 2002). In the similar human condition, metabolic syndrome is characterised by the development of obesity, IR, hypertension and dyslipidaemia. The extent to which hypertension and dyslipidaemia occur in affected horses has not been sufficiently explored to date. Nevertheless, IR-associated laminitis in obese horses represents a common problem with which veterinarians are presently faced. As is the case with the human condition, the development of obesity, IR and laminitis probably arises from a combination of management practices (inappropriately grain-laden ration combined with relative physical inactivity) in genetically susceptible individuals. Specific breeds that have been implicated for the development of IR include pony breeds,
Morgan horses, domesticated Spanish mustangs, European Warmbloods, and American Saddlebreds.

Insulin resistance (hyperinsulinaemia) is a common finding in horses affected with ECD. However, IR is not evident in all ECD-affected horses. It has been suggested that the prognosis for ECD is less favourable when IR is also present (Love 1993) and this observation was supported by the findings of McGowan et al. (2004). Whereas IR may be directly attributed to the direct action of increased GC effect in ECD, it is also likely that many horses are genetically affected with IR before they develop ECD. Genetic variability with respect to insulin action between individuals would mirror the situation in man and may predispose those individuals to the pathological consequences of IR over time (Yeni-Kmoshian et al. 2000). Under this assumption, underlying genetically based IR coupled with the development obesity probably increases the risk of developing laminitis, cardiovascular disease and PPID. Although IR is commonly diagnosed in mature horses and ponies affected with obesity and PPID, an understanding of the extent to which younger, normal horses are affected with differing degrees of IR is currently lacking.

Although GC may directly cause IR, it is also commonly present in association with obesity. Traditionally, adipocytes have been regarded as inactive repositories for energy storage. Remarkable new work demonstrates that there exist substantial differences between different adipocyte populations. Some adipocytes secrete endocrine signals that directly cause IR and hypertension (Lyon et al. 2003). Interestingly, in multiple species, it has been now shown that endocrinologically active adipocytes are especially plentiful in the omental and retroperitoneal fat tissue. Those human patients that have acquired intra-abdominal adiposity are at especially high risk for the development of IR, cardiovascular disease and diabetes. The numerous endocrine signals produced by adipocytes are collectively known as ‘adipokines’ (or adipocytokines) and include angiotensinogen, resistin, leptin, nonesterified fatty acids, adiponectin, and certain pro-inflammatory cytokines (including TNFα and IL-6) (Chaldakov et al. 2003). Increased production of adipokines by obese individuals is strongly associated with the development of IR; however, IR is not present in all obese individuals. Omental adipocytes are further characterised by the presence of the steroid-converting enzyme 11β-hydroxysteroid dehydrogenase-1 (11β-HSD-1) that acts to convert inactive cortisol (from the circulation) to active cortisol. Cortisol derived from adipose in this manner is believed to play a critical role in the pathogenesis of IR and metabolic syndrome (Masuzaki et al. 2001).

Both IR and laminitis are common findings in both obese horses (metabolic syndrome) and in horses affected with PPID. It is tempting to speculate that microvascular dysfunction arising from glucotoxic endotheliopathy could explain the development of laminitis in both of these cases. Alternatively, laminitis might result from a combination of IR and increased GC action (Johnson 2002).

In order to investigate any associations between IR, oxidative stress and the development of laminitis in horses affected with ECD, Keen et al. (2004) measured a battery of potential serum biochemical indices for vascular dysfunction, fibrin homeostasis, oxidative stress and glucose homeostasis. Although some evidence for a role of oxidative stress was identified (reduced plasma thiol levels), significant differences with respect to most of the other measured parameters were not identified. Lack of positive findings could be attributed to relative insensitivity of the assay procedures, the use of some assay kits that have not been validated for the horse, and lack of satisfactory case definitions resulting from the unavailability of an accurate antemortem diagnostic test for ECD.

The relationship between IR/MS and ECD is deserving of further investigation. Few would disagree that ECD-affected horses are commonly characterised by hirsutism (failure to shed out the haircoat), age, and poor bodily condition (muscle wasting and accretion of subcutaneous fat pads). Some typically affected horses have clearly developed laminitis and exhibit polyuria and polydipsia (PU:PD). Based on testing a large number of horses (including autopsy confirmation), the dexamethasone suppression test (DST) has been reported to be a very reliable test with very high sensitivity and specificity for ECD and is widely regarded as the ‘gold standard’ for antemortem diagnosis in horses (Dybdal et al. 1994). Although controversial, the contention that dexamethasone administration in the DST could be associated with risk of laminitis has led to the development of other tests for ECD. Specifically, demonstration of an elevated plasma e-ACTH concentration has been recommended to be as useful as the DST for diagnosis of ECD without the attending risk of laminitis (van der Kolk et al. 1995; Couteil et al. 1996). Using elevation of plasma e-ACTH concentration as the diagnostic criterion for ECD, it has been recognised that PPID may arise in relatively younger horses prior to the development of hirsutism and weight loss more commonly than had been previously recognised (Dybdal et al. 1994; Donaldson et al. 2002).

The authors have noted that there clearly exist exceptions to the diagnostic algorithm for ECD based on currently available tests (Keen et al. 2004). A separate endocrinological syndrome of mature horses characterised by insulin resistance, tissue specific cortisol dysregulation, obesity, and risk of laminitis has been postulated. This syndrome has been variably described as ‘insulin resistance syndrome’, ‘hypothyroidism’, ‘metabolic syndrome’, and ‘peripheral Cushing’s syndrome’ (Johnson 2002). As is the case for ECD, it has also been suggested that glucotoxic endotheliopathy, increased tissue-specific glucocorticoid action and oxidative stress are similarly essential for the development of laminitis in these cases. There are some important and noteworthy similarities between ECD and the metabolic syndrome. Moreover, the
fact that PPID sometimes occurs in relatively young horses that do not exhibit the ‘classic’ Cushingoid appearance requires that veterinarians should attempt to differentiate between the 2 syndromes with extreme caution. There exists currently a need for better syndrome definitions and diagnostic tests to facilitate accurate differentiation between the two.

We have hypothesised that the 2 conditions might be related. There is a substantial and increasing body of evidence in other species to support the contention that states of protracted IR lead to changes in the brain. Specifically, omental obesity may act to activate the hypothalamic-pituitary-adrenal axis directly through diverse neuroendocrinological mechanisms (Walker 2001). In the healthy state, insulin is secreted in response to a glycaemic meal and acts in the ventromedial hypothalamus to suppress appetite and inhibit the development of obesity (Lustig 2003). Whether IR is a cause or result of obesity is debated, although it is likely that both are true. Leptin is a specific adipokine that acts within the brain normally to suppress appetite. Failure of the leptin signal in the hypothalamus to suppress appetite (‘leptin resistance’) is also believed to contribute to the development of obesity through central mechanisms (Unger 2003). It has been suggested that leptin resistance is the most common cause of metabolic syndrome and obesity in other species and that, if an individual lives long enough, most people eventually develop leptin resistance in time (Unger 2003).

Neural mechanisms are also important in terms of communication between the adipose and the brain. Accumulation of lipid in the liver as a result of IR is signalled to the brain via the nucleus tractus solitarius through vagal afferents (Latour and Lautt 2002; La Fleur et al. 2003). Moreover, adipose tissue is innervated by both sympathetic nerves that act to stimulate lipolysis (releasing glycerol and free fatty acids) and by parasympathetic nerves that promote insulin sensitivity (Fliers et al. 2003). Interestingly, separate sets of parasympathetic neurons in the brain stem innervate either the visceral or the subcutaneous fat compartment. Therefore, it has been proposed that the CNS plays a major role in the hitherto unexplained regulation of body fat distribution (Fliers et al. 2003). Parasympathetic innervation is important for the maintenance of insulin sensitivity. All of the parasympathetic nerves that regulate hormonal control of insulin resistance pass through the cerebral vagus and its hepatic branch; transection at any of these sites leads to functional elimination of all hepatic parasympathetic input regulating insulin sensitivity. Insulin resistance is associated with attenuation of parasympathetic activity (Miller et al. 1999).

Pituitary dependent Cushing’s disease in horses arises as a result of a clonal expansion of POMC-secreting cells in the pars intermedia (Schott 2002). This situation is unique to the equine species and has been attributed to loss of dopaminergic inhibition of melanotropes in the pars intermedia resulting from primary hypothalamic perturbations (Schott 2002). Interestingly, a role for oxidative stress in loss of dopaminergic innervation of the pars intermedia from horses affected with ECD has been supported by work published recently (McFarlane et al. 2003). Changes in the hypothalamic-pituitary axis can be attributed to IR. We have hypothesised that, in many (if not all) cases, PPID (ECD) might represent an equine-specific complication of protracted IR and obesity in genetically susceptible individuals. In this paradigm, PPID might be identified in younger horses that do not exhibit the ‘traditional’ physical appearance of the old Cushingoid horse and yet the circulating plasma e-ACTH concentration might be elevated (incipient PPID).

Laminitis appears to be a potential complication of both metabolic syndrome and ECD. The extent to which these 2 endocrinological conditions are related is yet clearly deserving of further investigation. The development of laminitis as a result of IR, glucotoxic endotheliopathy and oxidative stress represents a potentially novel explanation for this ostensibly inflammatory condition under circumstances associated with heightened GC (anti-inflammatory) action.

In the future, in order to demonstrate bona fide differences between the healthy state and conditions associated with IR (including metabolic syndrome and ECD), it will be essential carefully to select age-matched normal horses that clearly do not fulfill any of the diagnostic criteria for either metabolic syndrome or ECD. The possibility that both MS and ECD represent different stages of the same underlying pathophysiological process (PPID) must also be clarified. In this regard, better agreement pertaining to syndrome definition between researchers should also be sought.

Broader recognition of the association between laminitis and glucotoxic endotheliopathy is warranted. The extent to which oxidative stress plays a role in the pathogenesis of endocrinopathic laminitis in metabolic syndrome and in PPID deserves further attention.

P. J. JOHNSON*, N. T. MESSER and V. K. GANJAM†
Departments of Veterinary Medicine and Surgery, and †Biomedical Sciences, College of Veterinary Medicine, University of Missouri, Columbia, Missouri 65211, USA.

References


Cushing’s syndromes, insulin resistance and endocrinopathic laminitis


