Received: 29 December 2022 | Accepted: 22 February 2023

DOI: 10.1111/evj.13932

EDITORIAL

Equine Veterinary Journal WILEY

Consequences of adiponectin deficiency: Can they be related to the pathophysiology of laminitis?

In the comprehensive review of laminitis molecular pathology (naturally occurring and experimental models) published in this issue,[1] the frequent finding of hypoadiponectinaemia as a neglected, potentially modifiable predisposing factor in naturally occurring laminitis was highlighted. There is much evidence from other species that adiponectin deficiency (relative or absolute) is an important factor predisposing to some of the complications of type-2 diabetes. It is tempting to speculate how some of the pathways activated by adiponectin could be involved in protecting against the development of the different forms of laminitis. This editorial uses data from laboratory animal experimental studies and studies of human patients to make such speculative connections, indicating a potential role of relative or absolute deficiency in circulating adiponectin in laminitis pathophysiology requires further study.

Comprehensive review of the complex biology of adiponectin and the research that underpins our current understanding can be found in the literature. [2,3] Produced by adipose tissue, adiponectin is recognised as an insulin sensitising agent providing communication between key tissues involved in homeostasis of metabolism (adipose tissue, skeletal muscle and liver). It is a key activator of the adenosine monophosphate-activated protein kinase (AMP-kinase) pathway, a cellular energy sensor and one of the two important pathways regulating energy balance.[4] AMP-kinase is activated when AMP/ATP ratio increases in a cell and the pathway it activates switches off energy utilising anabolic pathways in favour of catabolic pathways that generate ATP. AMP-kinase is also involved in regulation of cell growth, apoptosis and autophagy. As with any physiological homeostatic process, counter-balancing of AMP-kinase is the mammalian target of rapamycin (mTOR) signalling pathway,[5] promoting energy utilising anabolic pathways, including cell growth and replication and inhibiting autophagy.

In addition to the strong evidence that low adiponectin levels is a consistent risk factor associated with laminitis development and occurs concomitantly with the insulin dysregulation (ID) phenotype (see Elliott and Bailey 2023[1]), evidence from euglycaemic hyperinsulinaemic clamp (EHC) and sepsis-related laminitis (SRL) models of laminitis suggests signalling factors on the mTOR1C pathway, such as ribosomal S6 protein (RS6P) and AKt, are highly upregulated in the developmental phases (see Supplementary Table 2 in Reference [1]). In pasture-associated laminitis where ID and hypoadiponectinaemia are clear risk factors, the degree of activation of the mTORC1 pathway could be exacerbated as a result of a chronic imbalance of the counter-regulatory AMP-kinase pathway, activation of which is reduced with the relative deficiency of adiponectin. Activation of the mTORC1 pathway in pasture-associated laminitis is likely contributed by both insulin activation of insulin-like growth factor-1 (IGF-1) receptors and growth factor pathway activation resulting from platelet stimulation by lipopolysaccharide released from the gut as a result of carbohydrate overload (see Figure 4 in Reference[1]). The only model in which AMP-kinase activation has been studied is the dietary challenge model of endocrinopathic laminitis (EL) where phosphorylated AMP-kinase was reduced in the lamellae of lean ponies fed a high carbohydrate diet for 7 days.[6] The authors interpreted this finding as indicating the lamellar tissue was not energetically stressed in this hyperinsulinaemic model.

Currently, there is no evidence in the horse that hypoadiponectinaemia predisposes to poor outcomes, including laminitis, in horses with systemic inflammatory response syndrome (SIRS). Serum adiponectin concentrations have not been assessed as risk factors for SRL in equine SIRS patients and it would appear, clinically, that the phenotype of ID, which predisposes ponies to pasture-associated laminitis, does not lead to a higher prevalence of SIRs in these ponies than in the general equine population. However, there is mounting evidence from experimental animals and epidemiological studies in human clinical patients that adiponectin is protective in sepsis. For example, adiponectin gene knockout mice demonstrated eight-fold higher mortality than wild-type mice at 48 h following induction of the caecal ligation and puncture model.[7] In the same model, treatment with pioglitazone, a drug that upregulates adiponectin production, improved survival and inhibited the increase in pro-inflammatory cytokines and chemokines seen in this model.[8] Similarly, in rats undergoing caecal ligation and puncture to induce sepsis, exogenously administered adiponectin alleviated endothelial cell apoptosis and oxidative stress and inhibited the hypercoagulable state from being expressed in this model[9] and inhibited liver injury and hepatocyte apoptosis.[10] In human patients, higher pre-septic plasma adiponectin concentration was associated with improved survival[11] as was a greater rate of increase in plasma adiponectin concentration in the first 7 days following hospitalisation.[12] Thus, it appears that further investigation of the protective role of adiponectin in equine SIRS is warranted.

Hypoadiponectinaemia in other species has been shown to exacerbate the following pathological processes that may be of relevance to naturally occurring laminitis.

1 | EPITHELIAL CELL APOPTOSIS (PROGRAMMED CELL DEATH) AND PROLIFERATION

Adiponectin has well-characterised anti-apoptotic effects on myocardium and kidney tissue, which are often damaged as a result of the complications of metabolic syndrome and diabetes. In the kidney, for example, Zheng and Liu[13] found that adiponectin inhibited the rate of apoptosis development induced by $CoCl_2$ treatment (hypoxia-mimetic) in a cultured epithelial cell line from human kidney (HK2) cells. The same paper describes experiments involving adiponectin knockout mice and an ischaemic renal injury model. In vivo, apoptosis of kidney epithelial cells was higher in the knockout mice compared with wild type both under normal conditions and following induction of ischaemic injury in these mice. Apoptosis in the basal lamellar epithelial cells has been shown to occur in both naturally occurring laminitis (see Table 1 in Reference[1]) and in the developmental phase of EL and SRL models of laminitis (see Tables 1 and 2 in Reference [1]). [14] Hypoadiponectinaemia associated with increased laminitis risk could contribute to this increased susceptibility of lamellar epithelial basal cells (EBCs) to apoptosis.

Coupled with programmed cell death of damaged cells, proliferation of the remaining healthy cells occurs, a process that needs to be regulated to prevent excessive proliferation. The balance between cell death and proliferation in laminitis may also be influenced by mechanical forces resulting from the loss of adhesion of lamellar EBCs to the basement membrane. Many published studies have implicated adiponectin and the AMP-kinase pathway in inhibiting epithelial cell proliferation, and there is much interest in this aspect of cell biology in the pathogenesis of cancer and its relationship to obesity and insulin resistance. One example is a study that investigated the mechanism by which reduced circulating adiponectin concentrations were a risk factor for benign prostatic hypertrophy.[15] This study demonstrated that adiponectin receptor type 1 (Adipo-R1) was abundantly expressed on prostatic epithelial and stromal cells and, in vitro, adiponectin concentration dependently inhibited epidermal growth factor (EGF) stimulated proliferation and increasing apoptosis of these cells, effects that were inhibited by siRNA knockdown of Adipo-R1. Adiponectin activated AMP-kinase in these cells within 5 min of exposure with p38-MAP kinase activated after 15 min. EGF signalling was through the MEK pathway. In the same paper, a model of microscopic benign prostatic hypertrophy in mice was induced by a high fat diet (14 weeks) and was associated with reduced circulating adiponectin concentrations. Exogenous adiponectin in this model inhibited the microscopic prostatic hyperplasia. Thus, adiponectin is clearly involved in regulating growth and proliferation of epithelial cells, acting as a counterbalance to growth factors, ERK signalling and the mTOR pathway. It is therefore tempting to speculate that hypoadiponectinaemia associated with risk of laminitis influences the proliferative response of the lamellar EBCs to stressful/injurious stimuli, which results in an inappropriate proliferative response (see Tables 1 and 2 and Figure 4 in Reference [1]).

2 | ENDOPLASMIC RETICULUM STRESS

Adiponectin and the AMP-kinase pathway are thought to protect cells, including epithelial cells, from the phenomenon of endoplasmic reticulum stress (ER stress; leading to apoptotic cell death), which has been detected in lamellar epithelial cells of naturally occurring laminitis cases.[16] ER stress (also known as the unfolded protein response) is primarily stimulated by increased blood glucose and free fatty acid concentrations and has been coined 'the master regulator of metabolic syndrome'. [17]

An example of epithelial cell ER stress, which is thought to play a role in the complications of human metabolic syndrome and type 2 diabetes, occurs in podocytes. Specialised kidney epithelial cells forming the glomerular filtration barrier, podocytes, are damaged in diabetic nephropathy (and metabolic syndrome-induced kidney disease), giving rise to albuminuria and resulting in chronic kidney disease. Increased circulating levels of non-esterified fatty acids (in metabolic syndrome and type 2 diabetes) and reduced adiponectin concentrations are thought to play an important role in initiating podocyte stress and damage, whereas exogenous adiponectin is protective.[18] In vitro, palmitic acid (saturated fatty acid) stimulates ER stress in cultured podocytes through the generation of toxic metabolites, which leads to podocyte death. Activation of AMP-kinase in these cells, either through adiponectin or AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside), reduced palmitic acid-induced ER stress and cell death.[19]

3 | HYPOXIA AND OXIDATIVE STRESS

Adiponectin and the AMPK pathway it stimulates are important regulators of vascular function, particularly endothelial cell function (Rodríguez et al.).[4] It makes physiological sense that when energy in cells is low, the blood flow to those tissues should increase to supply more oxygen and nutrients to help them generate more ATP. The evidence that adiponectin plays a role in the protection against ischaemia and, through the AMPK pathway, protects against oxidative stress reducing reperfusion injury is very strong. Reduced circulating levels of adiponectin increase the risk of ischaemic vascular disease in people including stroke, myocardial infarction and kidney disease, all of which are complications of the metabolic syndrome and type 2 diabetes.[20,21] Much research, therefore, has been undertaken to understand this association. For example, Wang et al.,[22] demonstrated that adiponectin was partly responsible for the effect of ischaemic preconditioning (IPC), the phenomenon whereby a brief period of cardiac ischaemia is protective against a subsequent prolonged ischaemic episode. In experimental rats, they showed that IPC upregulated cardiac adiponectin expression markedly (2–2.5-fold at 6 and 12 h) with plasma adiponectin concentrations increasing by about 60% and peaking at 24 h. Cardiac knockdown of adiponectin, inhibition of AMP-kinase or cardiac knockdown of adiponectin receptors abrogated the beneficial effects of IPC (measured by less reduced ejection fraction and smaller area of ischaemic tissue following cardiac ischaemia),

which were restored by infusion of globular adiponectin. The precise source of cardiac adiponectin in these studies was not determined, although perivascular and epicardial adipose tissue is thought to actively produce adiponectin and perivascular fat-derived adipokines likely exert local effects on vascular function.[23]

Similar experimental evidence supports a direct role of adiponectin, signalling through AMP-kinase in protecting neuronal tissue[24] and kidney[25] from ischaemia-reperfusion injury, supporting the epidemiological evidence from human medicine that associates low plasma adiponectin concentrations with increased risk of stroke in patients suffering acute myocardial infarction.[26] Furthermore, obesity models in mice and rats lead to vascular dysfunction (endothelial dysfunction in particular) related to dysregulation of the AMP-kinase pathway,[27] which has been postulated to partly explain the link between obesity and non-diabetic chronic kidney disease in humans.

Exposure to high levels of insulin gives rise to vascular dysfunction of the equine digital vasculature. Here, direct effects of insulin on vascular tone change from vasodilation to vasoconstriction with prior exposure to high levels of insulin or blockade of the PI3-Kinase pathway. [28] Furthermore, vessels isolated from ponies with EL were shown to have endothelial cell dysfunction when compared with vessels from ponies with no evidence of EL.[29] The role of adiponectin in these phenomena has not been explored.

Much debate has occurred around the role of hypoxia and oxidative stress in the pathophysiology of laminitis over the last 30 years since the seminal work of Hood et al.,[30] proposed acute laminitis to be an ischaemia-reperfusion injury of the lamellae. Whilst hypoxia is a potential stressor on the metabolically active lamellar EBCs, most of the work using experimental models of SRL and EL has failed to demonstrate reduced perfusion or metabolic stress typical of hypoxia (increased lactate to pyruvate ratio, e.g.; see Table 5 in Reference [1]). The model studies suggest perfusion is increased and glucose and oxygen supply is not limiting for the epithelial cells, although very little experimental work has tested whether this is the case in chronically insulin-dysregulated ponies. The one exception is SLL, where increased HIF1α protein expression in lamellar EBCs[31] and lamellar interstitial fluid increased lactate to pyruvate ratio and reduced urea perfusion [32] support the occurrence of ischaemic hypoxia. It seems possible that dismissing ischaemic hypoxia as a potential stressor of digital epithelial cell function in ID ponies suffering from naturally occurring laminitis, where a component of supporting limb laminitis may be present, is premature based on the strength of evidence from other species of the direct association between adiponectin deficiency and AMPK pathway downregulation and ischaemic pathology.

In conclusion, relative or absolute lack of circulating adiponectin, which is an independent factor associated with risk of laminitis, could predispose to many of the pathological features of the different types of laminitis. Activation of adiponectin receptors would counterbalance the mTOR/RS6P pathway in EBCs, inhibiting epithelial cell endoplasmic reticular stress, which is potentially a key pathway involved in the pathogenesis of laminitis. Thus, based on knowledge in other species, the adiponectin/AMP-kinase pathway warrants further exploration in equine laminitis, particularly as options to therapeutically intervene by manipulating this pathway become available.

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