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ORIGINAL ARTICLE

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Short-term induced hyperinsulinaemia and dexamethasone challenge do not affect circulating total adiponectin concentrations in insulin-sensitive ponies

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Abstract

Background: Hypoadiponectinaemia is a risk factor for endocrinopathic laminitis, but the directionality and nature of its association with insulin dysregulation is unclear.

Objectives: To investigate the effects of short-term induced hyperinsulinaemia and dexamethasone challenge on circulating [total adiponectin] and whole blood expression of adiponectin (AdipoR1 and AdipoR2), insulin, and insulin-like growth factor 1 (IGF-1) receptors in insulin-sensitive ponies.

Study design: In vivo experiment.

Methods: Six never-laminitic, insulin-sensitive, native-breed UK ponies first underwent a dexamethasone challenge (0.08 mg/kg i.v.) with blood samples collected every 15 min over 3 h. After a 14-day washout period, hyperinsulinaemia was induced for 9 h via a euglycaemic–hyperinsulinaemic clamp (EHC), with blood samples collected every 30 min. Serum [insulin], plasma [total adiponectin], and plasma [IGF-1] were measured using validated assays and receptor gene expression was assessed via quantitative polymerase chain reaction (qPCR). Finally, whole blood was incubated with 10–1000 ng/mL dexamethasone for 3 h at 37°C to investigate its direct effects on gene expression.

Results: There were no adverse effects observed during either protocol. Dexamethasone challenge did not alter circulating [insulin] or [total adiponectin] at any timepoint, but significantly upregulated AdipoR1 and IGF-1R expression at 150 and 180 min. Ex vivo incubation of whole blood with dexamethasone did not alter expression of the genes examined. There was no change in [total adiponectin] or expression of the genes examined associated with EHC-induced hyperinsulinemia.

Main limitations: This was a small sample size that included only native-breed ponies; total adiponectin was measured rather than high-molecular-weight adiponectin.

Conclusions: Short-term induced hyperinsulinaemia and dexamethasone challenge did not affect circulating [total adiponectin] in insulin-sensitive ponies. However, dexamethasone administration was associated with upregulation of two receptors linked to adiponectin signalling, suggesting that a physiological response occurred

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possibly to counteract dexamethasone-associated changes in tissue insulin sensitivity.

KEYWORDS
adiponectin receptors, euglycaemic–hyperinsulinaemic clamp, horse, IGF-1, insulin dysregulation, insulin resistance

1 | INTRODUCTION

Insulin dysregulation (ID), the key feature of equine metabolic syndrome (EMS), may manifest as tissue insulin resistance (IR), basal hyperinsulinaemia, or an excessive insulin response to hydrolysable carbohydrates.[1] Previous studies have shown that continuous infusion of insulin via a euglycaemic–hyperinsulinaemic clamp (EHC) reliably causes endocrinopathic laminitis in healthy equids.[2,3] Although various histological and physiological changes associated with the actions of insulin have been reported in the lamellae of horses and ponies having undergone EHC,[4–8] the exact mechanisms through which short-term (48–72 h) hyperinsulinaemia induces laminitis remain unclear. In addition to ID, adipokine dysregulation is a feature of EMS that may be present in some animals.[9,10] Adiponectin is an insulin-sensitising adipokine that exists in various isoforms as globular adiponectin or as a full-length monomer that can form large complexes (high-molecular-weight [HMW] adiponectin).[11,12] Hypoadiponectinaemia is reported both before and after the development of endocrinopathic laminitis. Previously laminitic ponies have lower total adiponectin concentrations than never-laminitic ponies[13] and hypoadiponectinaemia was identified as an independent risk factor for the future development of endocrinopathic laminitis.[10,14] However, it is unclear whether there is a causal relationship between hypoadiponectinaemia and hyperinsulinaemia or other forms of ID.

Tissue IR is the impaired response of a target tissue to secreted insulin, which may or may not lead to compensatory hyperinsulinaemia.[15,16] In humans, this can be induced experimentally via administration of dexamethasone, which decreases glucose oxidation and uptake in skeletal muscle and adipose tissue, resulting in transient and reversible tissue IR.[17,18] In horses, repeated intravenous administration of dexamethasone over 21 days resulted in the development of IR and basal hyperinsulinaemia.[19,20] However, the short-term effects of intravenous dexamethasone administration on adiponectin regulation in equids have not yet been investigated. EHC is a well-established method used to induce basal hyperinsulinaemia and laminitis in horses.[2,3] Previous studies with human participants undergoing EHC reported both decreases[21–23] and increases[24] in adiponectin concentrations, leading to the hypothesis that EHC in ponies would cause measurable changes in plasma total adiponectin concentrations.

The primary aim of this study was to investigate the effects of short-term induced hyperinsulinaemia (via EHC) and dexamethasone challenge (via intravenous administration) on circulating total adiponectin concentrations in never-laminitic, insulin-sensitive, native-breed ponies. Total adiponectin concentrations were selected for measurement (rather than specific adiponectin isoforms) using the only commercial equine adiponectin assay currently available in the United Kingdom. Although HMW adiponectin is thought to be the most active form, measurement of total adiponectin is representative of diagnostic assays currently performed in equine clinical practice in the United Kingdom.

Cross-talk between insulin and insulin-like growth factor 1 receptor (IGF-1R) is thought to be implicated in the pathogenesis of laminitis through inappropriate stimulation of IGF-1R in lamellar tissue by excessive insulin concentrations.[25–28] In addition, cross-talk is known to occur between adiponectin and IGF-1R in humans in the context of cancer,[29,30] although this remains to be examined in the horse. Therefore, it was hypothesised that changes in circulating insulin or total adiponectin concentrations in response to hyperinsulinaemia and dexamethasone challenge may be accompanied by changes in the expression of the associated receptors. Thus, as a secondary aim, the RNA expression of adiponectin receptors (AdipoR1, AdipoR2, and T-cadherin), insulin receptor (INSR), and IGF-1R was investigated in whole blood during each protocol. A further investigation of the direct effects of corticosteroids on RNA expression from leucocytes was also undertaken by incubating blood samples with the relevant agent ex vivo, using methods previously described in human studies.[31]

2 | MATERIALS AND METHODS

2.1 | Animals

Previous work demonstrated that median total adiponectin concentrations (measured using a validated radioimmunoassay) in normal ponies are 3.72 (2.55–5.06) μg/mL.[10] Using these values, a sample size calculation assuming 80% power and $P = 0.05$ was performed using the University of British Columbia sample size calculator.[32] This suggested that a sample size of six animals would be sufficient to detect a reduction in [total adiponectin] of 25% or more in association with the induction of insulin dysregulation using a crossover design.

Six healthy native-breed ponies (two geldings, four mares; 6–18 years) with bodyweights (bwt) of 210–420 kg and ideal body conditions scores (4.5–5.5/9)[33] were therefore recruited to the study. Ponies had no previous history of laminitis and visual inspection of the hooves did not reveal any abnormalities consistent with previous episodes of laminitis. Animals also had normal basal insulin concentrations (mean ± SD = 2.92 ± 1.91 μIU/mL; measured on Immulite 2000 xpi) and normal insulin responses (29.36 ± 20.56 μIU/mL) to an

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oral sugar test performed with 0.45 mL/kg Karo light corn syrup.[34] No further dynamic insulin sensitivity testing was performed (e.g., combined glucose/insulin test [CGIT] or frequently sampled insulin-modified intravenous glucose tolerance test [FSIGTT]) before recruitment to the study. Adrenocorticotropic hormone concentrations (ACTH) were measured in animals aged >10 years and were within the season-adjusted reference range.[35] Animals showed no clinical signs of PPID or EMS.

Ponies were kept at pasture with access to sufficient grazing to maintain ideal bodyweight before and throughout the study period. Each pony underwent both administration of dexamethasone and EHC (to induce short-term hyperinsulinaemia), in this order with a 14-day washout period between protocols. Ponies were housed in pairs in separate but adjoining stables allowing physical contact during both procedures and had free access to hay and water throughout. Animals were not restrained in stocks during either protocol.

2.2 | Protocol 1: Dexamethasone challenge

A catheter (14-gauge, Milacath) was inserted into one jugular vein under local anaesthesia (2% lignocaine) and sutured in place. This catheter was used to administer dexamethasone (0.08 mg/kg bwt i.v.) and collect blood samples (10 mL) every 15 min over 3 h into both plain tubes (for serum) and EDTA-coated tubes (for plasma) to measure [insulin], [total adiponectin], and [IGF-1].

2.3 | Ex vivo stimulation of whole blood with dexamethasone

Residual samples from whole blood samples in EDTA tubes obtained from three of the ponies taken at time zero were taken to the lab within 1 h of collection. Each sample was aliquoted (500 µL) into four clean polypropylene tubes and incubated with 10, 100, or 1000 ng/mL dexamethasone or vehicle only (control) for 3 h at 37°C in a waterbath with gentle agitation. Samples were then immediately processed for measurement of gene expression via quantitative polymerase chain reaction (qPCR).

2.4 | Protocol 2: Euglycaemic–hyperinsulinaemic clamp

The method used was based on the EHC procedure by DeFronzo et al.[36] Briefly, bilateral jugular vein catheters were placed under local anaesthesia (2% lignocaine) and sutured in place. One catheter was used to administer insulin and glucose and the other was used to collect blood samples. Three baseline blood samples (10 mL each) were collected at 10-min intervals before the start of the infusions and results were averaged to determine mean basal glucose and insulin concentrations. A priming dose of insulin (Actrapid, Novo Nordisk; 45 µiU/kg bwt in 50 mL 0.9% saline) was administered as a bolus injection over 60 s.[36] The insulin infusion was then started at a steady rate of 6 µiU/min/kg bwt, which was maintained throughout the study. An infusion of glucose solution (50% w/v; Baxter) was also started at a rate of 24.0 mmol/min/kg bwt. Blood samples (1 mL) were collected every 5 min to monitor glucose concentrations, using a handheld glucometer (Accu-Chek Aviva 2, Roche) validated for equine samples,[37] until euglycaemia was reached (defined as blood glucose concentration maintained at 5.0 ± 1.0 mmol/L for 30 min without adjusting the infusion rate). Once in steady state, blood glucose concentrations were monitored every 30 min for 9 h and the glucose infusion rate was adjusted as necessary to maintain euglycaemia. During steady state, blood samples were collected every 30 min to measure [insulin] and [total adiponectin].

2.5 | Measurement of blood analytes

Blood samples were aliquoted into plain tubes and left to clot at room temperature for at least 30 min before centrifuging at 3000*g* for 5 min to obtain serum. Blood samples aliquoted into EDTA-coated tubes were inverted several times and placed on ice before centrifuging at 500*g* for 5 min to obtain plasma. Serum [insulin] and plasma [total adiponectin] were determined using automated commercial assays previously validated for horses at Liphook Equine Hospital Laboratory (Liphook, UK).[14,34,38] [IGF-1] was measured using an ELISA (Mediagnost) that was previously validated for use in equine plasma.[10] IGF-1 concentrations were not measured in samples from protocol 2 to avoid duplicating previous work.[25]

Data were assessed visually using histograms and a Shapiro–Wilk test was used. Data were approximately normally distributed. Analyte concentrations at each timepoint were compared with those at baseline using a repeated measures ANOVA, followed by Dunnett post hoc test. To determine differences between ponies, concentrations were compared using ANOVA followed by Tukey post hoc test comparing all ponies to each other. A mixed-effects model was used instead of ANOVA if there were any missing values, followed by Dunnett post hoc test. Geisser–Greenhouse correction was applied in cases where sphericity was violated. Analyses were performed using Prism v9.1.2 (GraphPad). Significance was accepted at $P \leq 0.05$.

2.6 | Measurement of gene expression using qPCR

Whole blood samples in EDTA tubes were stored at 4°C overnight before total RNA extraction using a PureLink™ RNA mini kit (Thermo Fisher Scientific), according to the manufacturer's instructions. RNA concentration and purity were assessed using a DS-11 Spectrophotometer (DeNovix) and cDNA was synthesised using a High-capacity RNA-to-cDNA™ kit (Thermo Fisher Scientific). Each reaction contained 10 µL RT buffer mix, 1 µL RT enzyme mix, 2 µg RNA template, and 7 µL PCR-grade water and the reaction proceeded in a thermocycler (SensoQuest) with the following protocol: 37°C, 60 min; 95°C, 5 min. cDNA was stored at −80°C until qPCR analysis.

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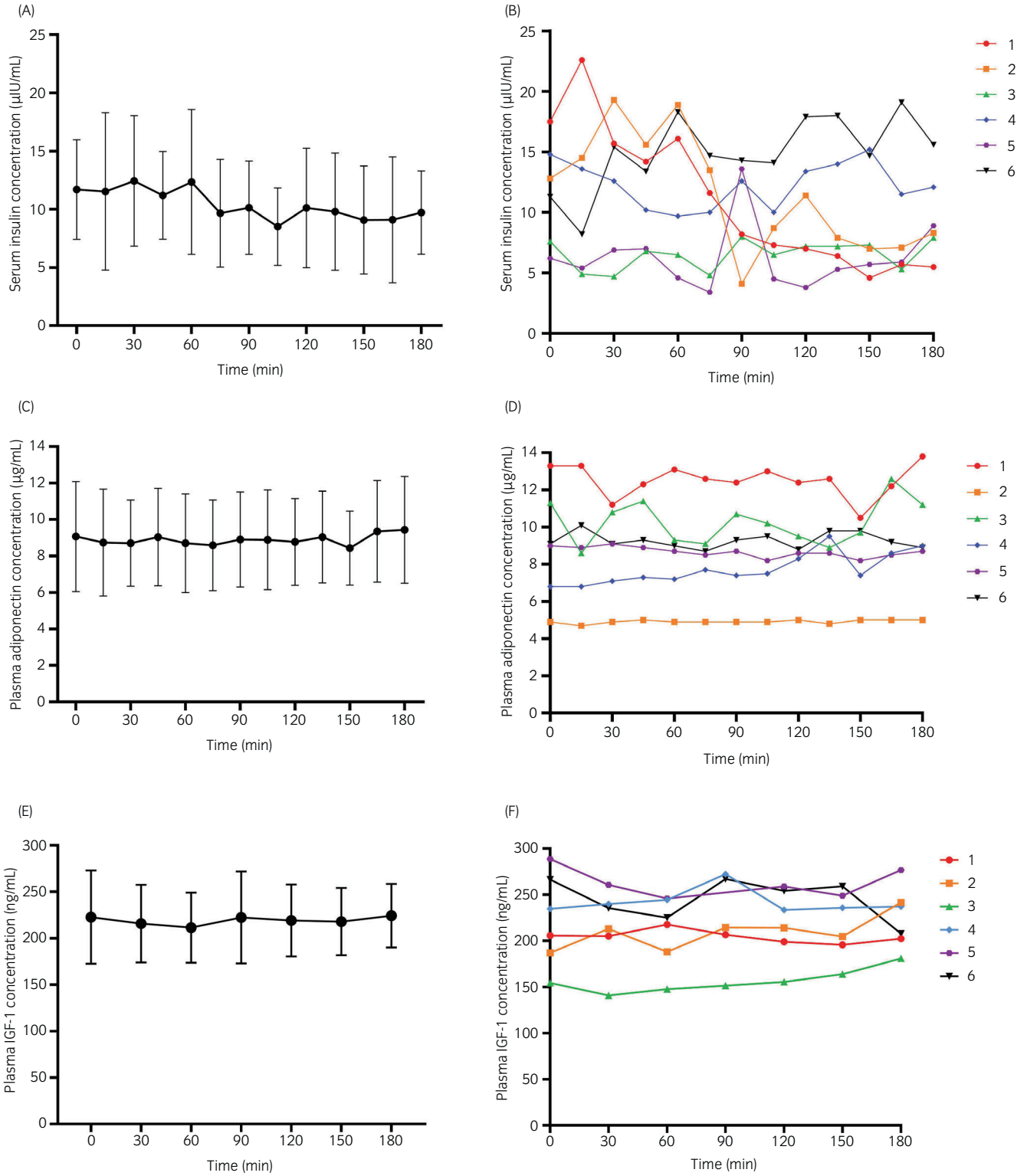

FIGURE 1 Serum insulin (A and B), plasma total adiponectin (C and D), and plasma insulin-like growth factor 1 (IGF-1; E and F) concentrations measured over 3 h after administration of dexamethasone (0.08 mg/kg via intravenous administration). A, C, E, mean concentrations ± SD for $n = 6$; B, D, F, concentrations for individual animals numbered 1–6.

qPCR was performed using the following TaqMan Gene Expression Assays (Thermo Fisher Scientific): AdipoR1, Ec01114954_m1; AdipoR2, Ec04320052_m1; T-cadherin, Ec03469102_m1; INSR, Ec04330427_m1; IGF-1R, Ec04330618_m1; GAPDH, Ec03210916_gH; β-actin, Ec04176172_gH. GAPDH and β-actin were included as housekeeping genes for normalisation and were duplexed

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to each target gene. Reactions (20 μL) comprised 10 μL TaqMan™ Fast Advanced Master Mix (Thermo Fisher Scientific), 1 μL of each gene expression assay (target and housekeeping gene), 6 μL PCR-grade water, and 2 μL template cDNA, and proceeded in a Bio-Rad CFX96 Touch Real-time PCR Detection System (Bio-Rad). Initial enzyme activation proceeded over 20 s at 95°C followed by 40 amplification cycles (denature: 3 s at 95°C, anneal: 30 s at 60°C). No-template negative controls containing water instead of cDNA were included in each qPCR run.

Expression of the target genes was normalised to that of GAPDH and β-actin using the $2^{-\Delta\Delta Ct}$ method.[39] RNA expression was expressed as log2 fold-expression relative to that at baseline. Expression at each timepoint was compared with that at baseline for each pony using a repeated measures ANOVA, followed by Dunnett post hoc test using GraphPad Prism v9.1.2. Significance was accepted at $P \leq 0.05$.

3 | RESULTS

3.1 | Animal experience

There were no adverse effects observed during or as a result of either protocol.

3.2 | Protocol 1: Dexamethasone challenge

3.2.1 | Blood analyte concentrations

There were no significant changes in serum insulin, plasma total adiponectin, or plasma IGF-1 concentrations measured over 3 h after dexamethasone administration compared with baseline (Figure 1).

3.2.2 | Gene expression

AdipoR1 and IGF-1R expression were significantly upregulated at 150 and 180 min after dexamethasone administration compared with that at baseline (Figure 2). There was no significant change in the expression of AdipoR2 or INSR (data not shown). The RNA expression of T-cadherin was undetectable in any of the samples collected.

3.2.3 | In vivo stimulation of whole blood with dexamethasone

There was no significant change in the RNA expression of AdipoR1 or IGF-1R in whole blood after 3 h incubation with 10–1000 ng/mL dexamethasone (Figure 3).

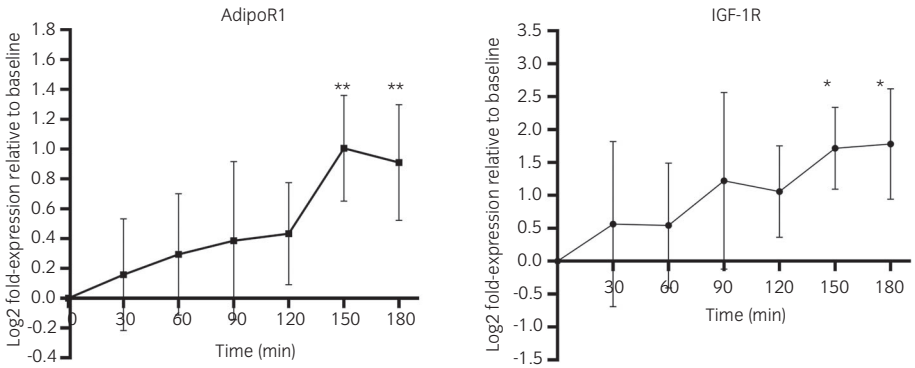

FIGURE 2 RNA expression of adiponectin receptor 1 (AdipoR1) and insulin-like growth factor 1 receptor (IGF-1R) in whole blood over 3 h after administration of dexamethasone. Data are shown as means ± SD ($n = 6$). *$P < 0.05$, **$P < 0.01$.

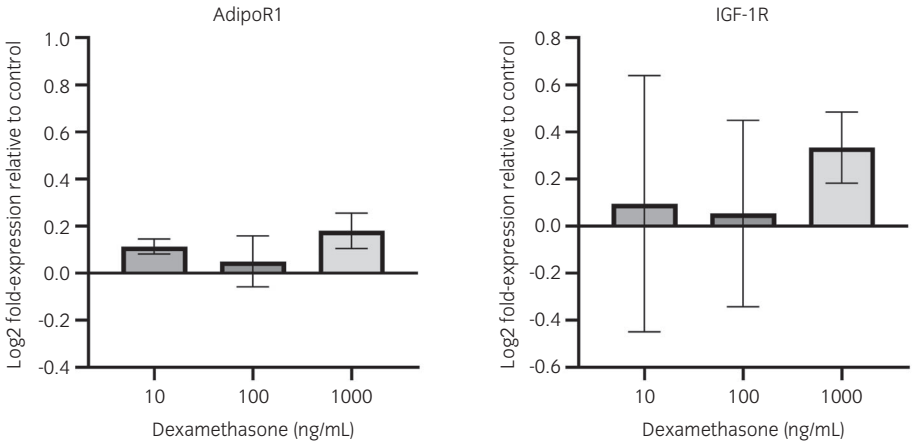

FIGURE 3 RNA expression of adiponectin receptor 1 (AdipoR1) and insulin-like growth factor 1 receptor (IGF-1R) in whole blood incubated ex vivo with 10, 100, and 1000 ng/mL dexamethasone for 3 h at 37°C. Expression relative to control (incubated with the vehicle only). Data are shown as means ± SD ($n = 3$).

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3.3 | Protocol 2: Euglycaemic–hyperinsulinaemic clamp

The mean ± standard deviation (SD) basal serum insulin concentration was 14.1 ± 6.8 μIU/mL. During the infusion, the mean ± SD steady-state insulin concentration reached was 544.2 ± 92.4 μIU/mL (Figure 4A,B). The overall maximum insulin concentration reached was 1500 μIU/mL and the mean ± SD maximum concentration for all ponies was 859.3 ± 316.4 μIU/mL. Plasma total adiponectin concentrations did not change significantly compared with baseline (Figure 4C,D).

In addition, there was no significant difference in the RNA expression of AdipoR1, AdipoR2, IGF-1R, or INSR during EHC compared with that at baseline (data not shown). Expression of T-cadherin was undetectable in equine whole blood.

4 | DISCUSSION

Circulating insulin concentrations were unchanged for up to 3 h following intravenous dexamethasone challenge in insulin-sensitive ponies. In humans, dexamethasone administration is reported to cause reversible IR, hyperinsulinaemia, and impaired glucose tolerance.[17,18] In previous studies conducted with Standardbred horses, repeated administration of dexamethasone over 21 days led to reduced insulin sensitivity and increased serum insulin concentrations.[19,20] In addition, a previous study showed that serum insulin concentrations increased 19 h after intramuscular administration of 0.04 mg/kg (overnight dexamethasone suppression test).[40] It is likely that administration of a single dose of dexamethasone in the present study was insufficient to increase insulin secretion, or that physiological changes induced by dexamethasone and subsequent tissue IR may take longer

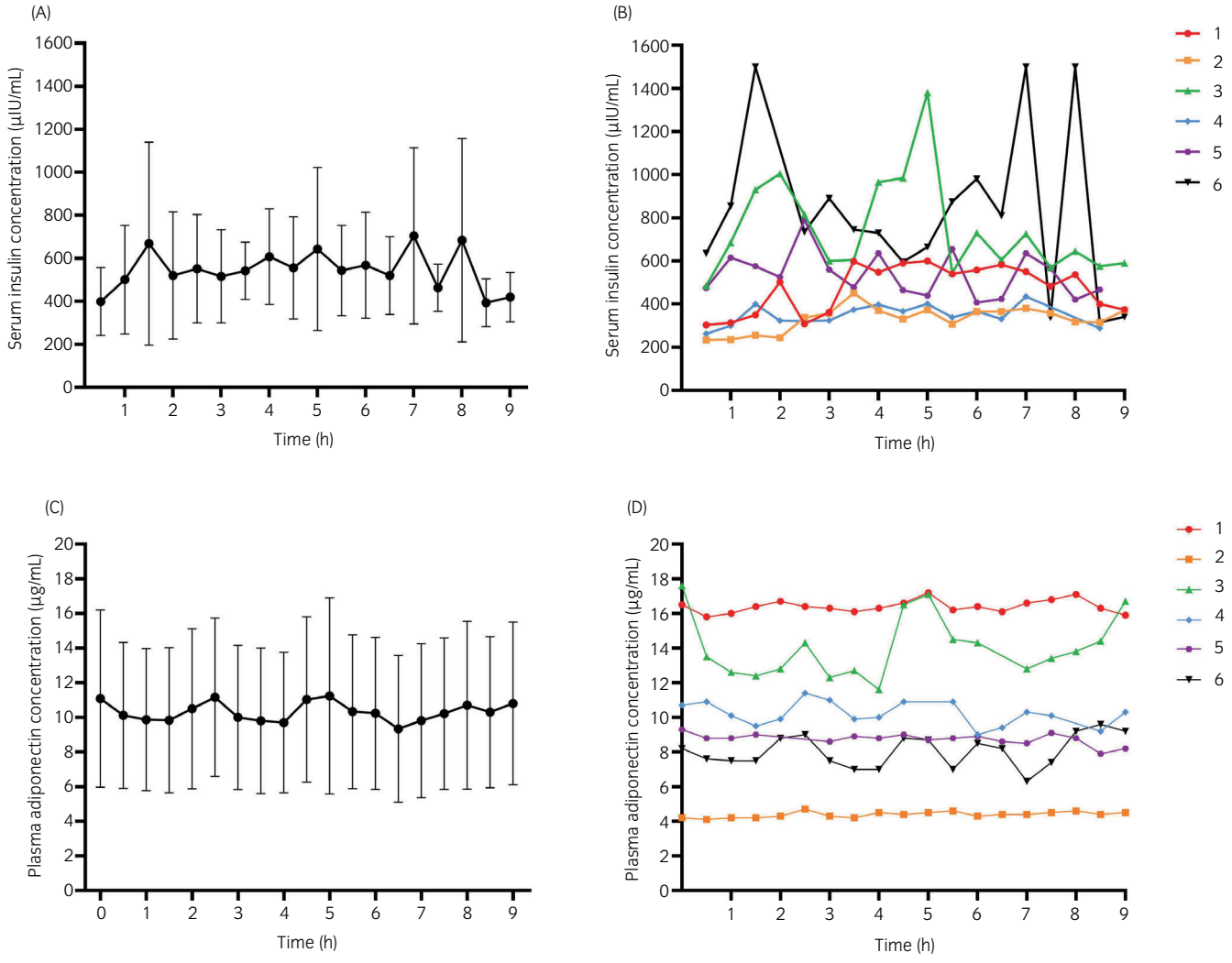

FIGURE 4 Serum insulin (A and B) and plasma total adiponectin (C and D) concentrations reached at steady-state during a 9-h euglycaemic–hyperinsulinaemic clamp. A and C, Mean concentrations ± standard deviation for $n = 6$; B and D, concentrations for individual animals numbered 1 to 6. Data for insulin and adiponectin were analysed using mixed-effects models as there were four and three missing values in these datasets, respectively, due to insufficient sample volume or samples undergoing haemolysis before analysis.

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than 3 h to manifest as increased insulin concentrations in circulation. Indeed, previous research in horses showed that insulin concentrations peaked 24 h after intravenous administration of the glucocorticoid triamcinolone acetonide.[41] In the present study, changes in insulin response may have occurred at the tissue level, although this was not investigated as it would have required more invasive sampling such as skeletal muscle tissue biopsy. To confirm the induction of tissue IR, the inclusion of dynamic insulin sensitivity tests, such as CGIT or FSIGTT, should be considered in future studies.

In rats, repeated dexamethasone administration over 3 days decreased serum adiponectin concentrations.[42] In humans, repeated dexamethasone treatment over 4 days increased total and HMW adiponectin concentrations.[43] There are no previous reports of the short-term effects of dexamethasone on adiponectin concentrations in ponies. However, a single dose of dexamethasone did not cause any changes in circulating total adiponectin concentrations in the present study. It is possible that the sampling period of 3 h may have been too short to observe changes in circulating adiponectin concentrations, as the effect of sampling time would be dependent on the clearance rate and half-life of adiponectin in equine plasma. If adiponectin secretion from adipocytes was reduced, its rate of removal from the circulation would determine how quickly the reduction in secretion would become apparent when measuring circulating concentrations. There are currently no data available regarding the half-life or clearance rate of adiponectin in equids. Results from the present study indicate that administration of a single dose of dexamethasone to healthy, insulin-sensitive ponies does not lead to measurable changes in either insulin or total adiponectin concentrations up to and including 3 h following.

In contrast, differences in AdipoR1 and IGF-1R gene expression were observed in response to dexamethasone administration. To confirm that these changes were not associated with the direct effect of dexamethasone on circulating leucocytes, whole blood was incubated with dexamethasone at concentrations similar to those expected after intravenous administration of 0.08 mg/kg dexamethasone.[44] There was no change in the expression of AdipoR1 or IGF-1R in response to dexamethasone treatment of ex vivo whole blood. Upregulation of these two genes may therefore be associated with the systemic effects of dexamethasone. Alternatively, this may be due to natural variation in the expression of AdipoR1 and IGF-1R related to the circadian rhythm. The expression of AdipoR1 (and AdipoR2) is subject to diurnal variation in human adipose tissue[45] and in mouse liver and adipose tissue,[46,47] but there are no previous reports of circadian variation in the expression of AdipoR1 and IGF-1R in equine whole blood. Furthermore, the sampling period was only 3 h and it is unlikely that the significant changes in expression observed would be due to normal diurnal variation.

Our results therefore indicate that administration of dexamethasone causes upregulation of both AdipoR1 and IGF-1R in the short term, which may be associated with changes in insulin sensitivity. Upregulation of a receptor would be expected if the associated ligand was downregulated. Upregulation of AdipoR1, which is the main receptor for globular adiponectin,[48] may therefore suggest changes in globular adiponectin production in adipocytes, although this may not have been evident when measuring plasma total adiponectin and would require further investigation. Total adiponectin was measured in the present study and changes in the composition of adiponectin isoforms were not investigated as no validated assay is currently available in the United Kingdom to measure these.

This is the first study to investigate the expression of adiponectin, insulin, and IGF-1 receptors in equine blood, a convenient sample that can be obtained easily via minimally invasive jugular venepuncture. The expression of adiponectin receptors in subsets of peripheral immune cells has previously been investigated in humans.[49] Studies in human subjects have also reported the differential expression of AdipoR1, AdipoR2, and T-cadherin (a non-classical receptor for full-length adiponectin) in specific populations of peripheral blood mononuclear cells and its association with immune deficiencies.[50] The expression of AdipoR1 and AdipoR2 was also investigated in peripheral monocytes in obese and overweight patients with coronary artery disease[51] and in leucocytes in children with hypertension[52] and obesity.[53] Human studies have therefore shown a relationship between circulating adiponectin concentrations in the blood, the expression on adiponectin receptors on immune cells, and various disease states. As this has not yet been investigated in the horse, it is a novel and interesting research lead in equine metabolic syndrome. In the present study, we describe reproducible methods for the measurement of gene expression in the blood. This includes the use of commercially available primers and probes, which were selected to facilitate standardisation and reproducibility for future use by other researchers. However, it must be noted that changes in RNA expression do not necessarily imply changes in protein expression, which was not investigated here. Furthermore, there are very few data regarding the relationship between the expression of adiponectin receptors in human blood (peripheral leucocytes) and other tissues (such as adipose or liver tissue) or cell types[54] and this is yet to be investigated in the horse.

Next, hyperinsulinaemia was induced for 9 h via EHC, with the hypothesis that adiponectin concentrations would change in response to steady-state hyperinsulinaemia. In contrast to previous results from human subjects, the present study showed that EHC-induced hyperinsulinaemia did not cause any changes in total adiponectin concentrations in ponies. Human studies have reported both increased and decreased adiponectin concentrations in subjects undergoing EHC, likely due to differences in the participants' health status (e.g., presence of obesity and type 2 diabetes mellitus). Indeed, EHC-induced hyperinsulinaemia caused a decrease in total adiponectin concentrations in lean but not in obese individuals.[24] Another study conducted in human subjects reported differences in the concentrations of different adiponectin isoforms in diabetic patients.[23] HMW adiponectin concentrations were specifically decreased during EHC whereas other adiponectin isoforms were unaffected.[23] There are several potential reasons for the differences in observations reported in humans and ponies, including methodological differences relating to the sampling period (9 h in the present study vs. 3.5–7 h in previous studies with human subjects[22–24]) and the form of adiponectin measured (total vs. HMW adiponectin).

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Previous studies have reported hypoadiponectinaemia both before[10] and after[13] the advent of laminitis, indicating this observation may be both a risk factor for and a consequence of laminitis. However, the results from the present study indicate that hypoadiponectinaemia is not directly caused by short-term tissue IR or hyperinsulinaemia. The relationship between adiponectin and insulin appears to be bi-directional, as a previous study showed that increasing HMW adiponectin concentrations in equids via treatment with pioglitazone also decreased insulin concentrations in response to an oral sugar test.[55] Similarly, dietary supplementation with resveratrol and leucine increased HMW adiponectin concentrations and decreased both basal and post-prandial insulin concentrations.[56] The relationship between adiponectin and insulin sensitivity is undoubtedly complex in the whole animal and involves multiple feedback systems that may be influenced by chronic conditions such as obesity and IR. The approach used in the present study involved only very defined and limited interventions, which would have reduced the impact of some of these other factors. It is, therefore, important to acknowledge that a whole systems approach in which the entire insulin–adiponectin system is investigated and modelled would produce a more complete picture of the in vivo situation.

This study has several limitations, not least the small sample size and the fact that only native UK pony breeds were represented. Although these ponies were purposefully selected to represent the most common breeds affected by EMS and laminitis in the United Kingdom, this does limit the applicability of findings to other study populations. Furthermore, these ponies were healthy, insulin-sensitive, and had no previous history of laminitis. The aim of the study was to examine the effect of administration of a physiological antagonist to insulin on adiponectin in normal, healthy ponies and the inclusion of ponies with pre-existing insulin dysregulation would not have been representative of the normal physiological response to insulin antagonism. In addition, these inclusion criteria were used to reduce the risk of inducing laminitis during study protocols. However, this does mean that findings may not be generalisable to equids with insulin dysregulation or previous episodes of laminitis, or to horses as this study was conducted in ponies specifically. In addition, only total adiponectin was measured, rather than specific isoforms. HMW adiponectin is considered the most biologically active form, although little is known about the roles and functions of other adiponectin isoforms in the horse. Although validated assays are available in other countries to measure equine HMW adiponectin, these are not available in the United Kingdom. Therefore, the only assay available to the authors was an immunoturbidimetric assay (offered and validated in-house by Liphook Equine Hospital), which measures total adiponectin. Ideally, this study would have included the determination of [total adiponectin] and adiponectin sub-fractions, including [HMW adiponectin].

In conclusion, short-term induced hyperinsulinaemia and dexamethasone challenge did not affect circulating total adiponectin concentrations in healthy, insulin-sensitive ponies. However, intravenous dexamethasone administration was associated with upregulation of AdipoR1 and IGF-1R, two receptors linked to adiponectin signalling, which may suggest a physiological response aiming to increase tissue sensitivity to adiponectin. Hypoadiponectinaemia does not appear to be a direct consequence of short-term hyperinsulinaemia or dexamethasone administration, but the effect of longer-term ID and the third form of ID (excessive insulin responses to non-structural carbohydrates) on adiponectin signalling, as well as the distribution of different adiponectin isoforms and their response to insulin, requires further research.

AUTHOR CONTRIBUTIONS

Marine A. Barnabé and Nicola Menzies-Gow contributed to study design, study execution, data analysis and interpretation, preparation of the manuscript, final approval of the manuscript and takes responsibility for the integrity of the data and the accuracy of data analysis. Jonathan Elliott and Patricia A. Harris contributed to study design, data interpretation, preparation of the manuscript, and final approval of the manuscript.

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CONFLICT OF INTEREST STATEMENT

P. Harris is an employee of Waltham Petcare Science Institute.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/evj.14012.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/record/8338547, reference number 8338547.

ETHICAL ANIMAL RESEARCH

This study was approved by the Royal Veterinary College Animal Welfare and Ethical Review Board (2020-135N) and the Clinical Research Ethical Review Board (URN 2020-2003-2) and was conducted under a UK Home Office licence (PP5634400).

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