Influence of dietary restriction and low-intensity exercise on weight loss and insulin sensitivity in obese equids

Nicholas J. Bamford | Samantha J. Potter | Courtnay L. Baskerville | Patricia A. Harris | Simon R. Bailey

1Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Werribee, Victoria, Australia
2Equine Studies Group, WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, United Kingdom

Correspondence
Nicholas Bamford, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Corner Park Drive and Flemington Road, Parkville, Victoria 3010, Australia.
Email: n.bamford@unimelb.edu.au

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Background: The importance of including exercise with dietary modification for the management of obese equids is not clearly understood.

Objectives: To evaluate the effect of a practical low-intensity exercise regimen, in addition to dietary restriction, on indices of insulin sensitivity (SI) and plasma adipokine concentrations in obese equids.

Animals: Twenty-four obese (body condition score [BCS] ≥ 7/9) horses and ponies.

Methods: Over a 12-week period, animals received either dietary restriction only (DIET) or dietary restriction plus low-intensity exercise (DIET+EX). All animals were provided with a restricted ration of grass hay at 1.25% body weight (BW) on a dry matter basis, providing 82.5% estimated digestible energy requirements. The DIET+EX group undertook low-intensity exercise 5 days per week on an automated horse walker. Before and after weight loss, total body fat mass (TBFM) was determined, indices of SI were calculated using minimal model analysis of a frequently sampled IV glucose tolerance test, and adipokines plus inflammatory biomarkers were measured using validated assays.

Results: Decreases in BCS, BW, and TBFM were similar between groups (all \( P > .05 \)). After weight loss, animals in both groups had decreased basal insulin and leptin concentrations, and increased adiponectin concentrations (all \( P < .001 \)). Furthermore, animals in the DIET+EX group had significantly improved SI and decreased serum amyloid A concentrations relative to animals in the DIET group (both \( P = .01 \)).

Conclusions and Clinical Importance: Regular low-intensity exercise provided additional health benefits compared with dietary restriction alone in this population of obese equids.

KEYWORDS
adipokine, equine metabolic syndrome, horse, inflammation, laminitis, nutrition

1 INTRODUCTION

The clinical clustering of obesity and insulin dysregulation, termed equine metabolic syndrome (EMS), is linked to the development of laminitis in horses and ponies. The prevalence of obesity has reached alarming rates within domestic equine populations in recent times. A lack of effective treatments for laminitis suggests that preventative countermeasures need to be instituted for animals with EMS before the onset of disease. Current recommendations for the management of animals with EMS are directed at weight loss and alleviating insulin dysregulation. Dietary modification is the cornerstone of an effective management program, with caloric restriction essential to achieve weight loss and control of nonstructural carbohydrate (NSC) intake is key to mitigating hyperinsulinemia.
Weight loss and improved glucose tolerance or tissue insulin sensitivity (SI) for animals with EMS has been demonstrated using various protocols of dietary restriction, with or without prescriptive exercise.10–17 The addition of exercise is recommended for horses and ponies that presently are not lame.1,17,18 However, previous work in equids has yielded conflicting results regarding the benefit of exercise, which could stem from differences in research methodology (eg, diet, exercise regimen, and method of SI assessment) and the characteristics of horses studied (eg, breed, level of adiposity, and degree of insulin dysregulation).19–25 Exercise without dietary modification might be insufficient to improve SI in equids despite inducing weight loss,21,24 although exercise could provide other health benefits, including decreased biomarkers of systemic inflammation such as serum amyloid A (SAA) concentration.23

Controlled studies that separate the effects of dietary restriction and exercise have been recommended to further appraise the benefits of exercise for animals with EMS.17 Our study aimed to evaluate the addition of a practical low-intensity exercise regimen to a program of dietary restriction in a group of obese horses and ponies. Assessed outcome variables included measures of adiposity, indices of SI, and plasma concentrations of adipokines and biomarkers of systemic inflammation. The null hypothesis was that regular low-intensity exercise in combination with dietary restriction would not provide additional health benefits over dietary restriction alone.

2 | MATERIALS AND METHODS

2.1 | Animals and study design

Twenty-four equids were studied, including 8 Standardbred horses (9 ± 2 years; 530 ± 30 kg; 5 geldings, 3 mares), 8 mixed-breed ponies (9 ± 3 years; 350 ± 60 kg; 5 geldings, 3 mares), and 8 Andalusian-Cross horses (8 ± 2 years; 550 ± 70 kg; 3 geldings, 5 mares). All animals were in obese body condition as defined by body condition score (BCS ≥ 7/9) at the study outset and had been so for between 4 and 8 weeks before the start of the study. Twenty-two animals were enrolled previously in a study of diet-induced weight gain;26 2 others (1 Standardbred and 1 pony) from the study of diet-induced weight gain were replaced for reasons unrelated to the study protocol. The 2 replacement animals had been kept in an adjacent dry lot paddock for the previous 6 months with similar daily husbandry procedures. All animals had remained sedentary (no prescriptive exercise) for at least the previous 12 months. An a priori estimate of study power indicated that 10 animals per group were appropriate to detect a 1-unit change in SI with a power of 80% (alpha = 0.05, 2-tailed). The use of animals was approved by the University of Melbourne Animal Ethics Committee (ID: 1011918).

The horses and ponies underwent an initial acclimation period of 4 weeks, during which they were kept in large dry lot paddocks and provided with ad libitum access to mixed grass hay and fresh water. A small meal containing lucerne chaff (0.5 g/kg body weight [BW], as fed) and a vitamin/mineral powder (60 mg/kg BW; Ranvet, East Botany, New South Wales, Australia) was provided 5 days per week, with animals moved to individual yards (4 × 4 m) along the perimeter of the dry lot during meal times.

The weight loss program lasted 12 weeks. Horses and ponies were blocked by both breed and the diet group to which they were assigned in the previous study of diet-induced weight gain. They were then randomly assigned to 1 of 2 intervention groups (such that each group contained 12 animals; 4 of each breed): dietary restriction only (DIET) or dietary restriction plus low-intensity exercise (DIET+EX). Animals spent most of each day in separate yards (4 × 4 m) to ensure individual feed intake, with group turnout into large dry lot paddocks (no forage available) occurring between 1000 and 1600 hours each day. All animals were provided with a restricted ration of the same batch of mixed grass hay (Supporting Information Table S1) at 1.25% BW (dry matter [DM] basis) daily (7.5 MJ/kg DM, 9.1% NSC) that was divided and fed at 0700 hours (one-quarter), 1600 hours (one-quarter), and 1900 hours (one-half). Hay was placed in a commercial feeder covered with a small-holed steel mesh to slow the rate of intake. A single daily meal containing soybean meal (1.0 g/kg BW, as fed), lucerne chaff (1.0 g/kg BW, as fed), and a vitamin/mineral powder (60 mg/kg BW; Ranvet) was provided at 0900 hours. The rationale for the daily meal was to ensure adequate dietary protein intake and minimize muscle protein degradation during the period of dietary restriction.12,15 The total ration offered 11.5 MJ/100 kg BW, providing 82.5% of estimated daily digestible energy requirements.27 Individual feed provisions were adjusted weekly according to changes in BW.

The DIET+EX group was subjected to a low-intensity exercise program using an automated horse walker (Priefert, Scone, New South Wales, Australia) on a surface of soil and sand (1:1 ratio). Animals in this group were trained to use the horse walker during the final 2 weeks of the acclimation period. During the 12-week weight loss program, exercise occurred 5 days per week between 0800 and 1000 hours, and consisted of 5 minutes walking, 15 minutes brisk trotting (2.0 m/s for ponies, 3.0 m/s for smaller Andalusians, and 3.5 m/s for Standardbreds and larger Andalusians), and 5 minutes walking. This protocol was developed after seeking feedback from horse owners regarding practicality (data not shown) and was similar to previously described programs of low-intensity exercise for equids.15,16 It was determined that dietary restriction would be terminated for any animals that reached BCS ≤ 5/9, with the amount of hay increased to meet maintenance digestible energy requirements until the end of the study period.

2.2 | Assessment of adiposity

Adiposity was assessed before and after dietary restriction occurred. Body weight was measured using calibrated scales (Horse Weigh, Penybont, United Kingdom). Body condition score and cresty neck score (CNS) were assessed by an experienced observer using the modified Henneke (1-9) scale and Carter (0-5) scale, respectively.28–30 Total body fat mass (TBFM) was determined by a heavy isotope dilution method as previously described.31 Briefly, animals were weighed and a baseline blood sample was collected immediately before 0.12 g/kg BW deuterium oxide was administered IV through a temporary jugular catheter. Syringes were weighed (±0.01 g) to accurately determine the actual dose delivered. After a 4-hour
equilibration period, a second blood sample was drawn from the contralateral jugular vein. Plasma deuterium oxide concentrations in both samples were measured with gas isotope ratio mass spectrometry (Iso-Analytical, Crewe, United Kingdom), with calculations to derive TBFM performed as previously described.31

2.3 | Assessment of SI

Insulin-modified frequently sampled IV glucose tolerance tests (FSIGT) were performed in all animals over 5 days, before the start of the acclimation period and after the period of dietary restriction. A standard FSIGT procedure for equids was followed as previously described.32 Animals were allowed to have until 1 hour before commencement of the FSIGT and water throughout. Briefly, between 0800 and 0830 hours, a catheter was placed in the left jugular vein under local anesthesia and blood samples were collected 60 minutes, 45 minutes, and immediately before a glucose bolus (300 mg/kg BW; 40% weight/volume) administered over 2 minutes through the jugular catheter, then irrigated thoroughly with 0.9% saline solution. Twenty minutes later, a rapid insulin bolus (20 mU/kg BW; Actrapid, Novo Nordisk, Bagsvaerd, Denmark) was delivered via the right jugular vein. Blood samples were collected 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes after the glucose bolus. Samples were transferred to tubes containing lithium heparin anticoagulant (BD Vacutainer, Plymouth, United Kingdom) and placed on ice until centrifugation (1000 g at 4°C for 10 minutes), with separated plasma stored at −80°C until analysis.

2.4 | Blood collection and plasma measurements

Blood samples were collected from each animal before the morning meal, and before and after the period of dietary restriction. Blood samples were transferred to tubes containing lithium heparin or EDTA anticoagulant (BD Vacutainer) and kept on ice until centrifugation (1000g at 4°C for 10 minutes), with separated plasma stored at −80°C until analysis.

Glucose was measured using a colorimetric assay (Cayman Chemical Co, Ann Arbor, Michigan). Insulin (Coat-A-Count, Siemens Diagnostics, Los Angeles, California), leptin (Coat-A-Count), high-molecular weight adiponectin (Millipore, Billerica, Massachusetts), tumor necrosis factor-α (Thermo Fisher Scientific, Scoresby, Victoria, Australia), and SAA (Tridelta Development, Maynooth, Ireland) were measured using assays previously validated for equine samples.33–37

2.5 | Data analysis

Minimal model analysis of glucose and insulin curves from the FSIGT was performed by MinMod Millennium computer software (Version 6.02, University of Pennsylvania, Kennett Square, Pennsylvania). Values for SI, acute insulin response to glucose (AIRg), disposition index (DI; SI × AIRg), and glucose effectiveness were obtained.38

Statistical analysis was performed by SPSS (Version 22, IBM, New York, New York) and GraphPad Prism (Version 6.0, GraphPad, La Jolla, California). Measured outcome variables with repeated measures were analyzed using a mixed-model analysis of variance (ANOVA), factoring the effects of time (before and after dietary restriction), breed group, and intervention group (DIET or DIET+EX). Individuals were nested within intervention as a random effect. Data were assessed for normality by the Shapiro-Wilk test, and Levene's test was used to evaluate homogeneity of variances. When significant 3-way or 2-way interactions were present, these were further explored using simple 2-way or simple main effects as appropriate. Pairwise comparisons were performed by Fisher's least significant difference test. Body weight was analyzed as percentage change from starting values to account for heterogeneity in body mass among breeds using a mixed-model ANOVA that included the effects of breed and intervention group. Data were reported as mean ± SD. Significance was accepted at P < .05.

3 | RESULTS

3.1 | Animals

No signs of ill health or lameness were observed in any of the animals studied. No feed refusals were noted at any time, and all animals assigned to the DIET+EX group participated in every session of prescriptive exercise throughout the study.

3.2 | Adiposity

Body weight decreased by 6.2 ± 4.3% for the DIET group and 7.7 ± 2.1% for the DIET+EX group (P = .30). Significant decreases in BCS, CNS, and TBFM occurred relative to starting values for both groups (Table 1; all P < .001). A main effect of breed was present for CNS, with Standardbreds having lower scores relative to ponies and Andalusians at both time points (P = .003). A significant 3-way interaction was detected for TBFM (P = .04), with ponies exhibiting lower TBFM within the DIET+EX group when compared to ponies in the DIET group after weight loss (P = .01).

### Table 1. Adiposity (mean ± SD) of equids subjected to a 12-week weight loss program consisting of dietary restriction alone (DIET; n = 12) or dietary restriction plus low-intensity exercise (DIET+EX; n = 12)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before weight loss</th>
<th>After weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS (1-9 scale)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>7.6 ± 0.6a</td>
<td>6.1 ± 1.1b</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>7.5 ± 0.5a</td>
<td>5.8 ± 0.6b</td>
</tr>
<tr>
<td>CNS (0-5 scale)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>3.4 ± 0.7a</td>
<td>2.7 ± 0.8b</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>3.4 ± 0.5a</td>
<td>2.5 ± 0.7b</td>
</tr>
<tr>
<td>TBFM (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>17.1 ± 3.0a</td>
<td>12.3 ± 3.2b</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>16.3 ± 1.5a</td>
<td>10.8 ± 1.6b</td>
</tr>
</tbody>
</table>

Different superscript letters indicate significant difference between groups or time points for each variable (P < .05). Abbreviations: BCS, body condition score; CNS, cresty neck score; TBFM, total body fat mass.
3.3 | Insulin sensitivity

Results of the FSIGT are shown in Table 2 and Figure 1. A significant 2-way interaction between time and intervention group was detected for SI (P = .009). Values for SI were similar between intervention groups before weight loss (P = .34), but animals in the DIET+EX group had higher values than those in the DIET group after weight loss occurred (P = .01). A main effect of breed was present for both SI and AIRg, with Standardbreds having higher SI values and lower AIRg values relative to ponies and Andalusians at both time points (P = .001 and .009, respectively). A significant 2-way interaction between time and intervention group was detected for DI (P = .005). Values for DI were similar between intervention groups before weight loss (P = .60) but were higher in the DIET+EX group after weight loss compared to the DIET group (P < .001).

3.4 | Plasma analytes

Results of plasma analyses are shown in Table 3. A significant effect of time was detected for several plasma analytes, with both intervention groups having lower basal insulin and leptin concentrations (both P < .001), and higher adiponectin concentrations (P < .001), after weight loss. A significant 2-way interaction between time and intervention group was detected for SAA (P = .04). Intervention groups were similar before weight loss (P = .42), but SAA concentrations were lower in the DIET+EX group after weight loss compared to the DIET group (P = .01).

4 | DISCUSSION

Our results suggest that regular low-intensity exercise provides additional health benefits when combined with dietary restriction as part of a weight loss program for obese equids. At the end of a 12-week weight loss program, animals in both the DIET and DIET+EX

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Frequently sampled intravenous glucose tolerance test results (mean ± SD) of equids subjected to a 12-week weight loss program consisting of dietary restriction alone (DIET; n = 12) or dietary restriction plus low-intensity exercise (DIET+EX; n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Before weight loss</td>
</tr>
<tr>
<td>SI (x10⁻⁵/[mIU·min])</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>2.21 ± 1.21a</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>1.88 ± 1.31a</td>
</tr>
<tr>
<td>AIRg (mIU·min⁻¹/L)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>416 ± 286</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>415 ± 244</td>
</tr>
<tr>
<td>DI (x10⁻⁷)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>721 ± 343a</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>641 ± 335a</td>
</tr>
<tr>
<td>Sg (x10⁻²/min)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>1.91 ± 0.54</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>2.24 ± 0.89</td>
</tr>
</tbody>
</table>

Different superscript letters indicate significant difference between groups or time points for each variable (P < .05). Abbreviations: AIRg, acute insulin response to glucose; DI, disposition index; Sg, glucose effectiveness; SI, insulin sensitivity.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Plasma analytes (mean ± SD) of equids subjected to a 12-week weight loss program consisting of dietary restriction alone (DIET; n = 12) or dietary restriction plus low-intensity exercise (DIET+EX; n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Before weight loss</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>6.8 ± 2.8a</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>6.3 ± 2.0a</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>7.0 ± 1.8a</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>7.5 ± 2.2a</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>2.2 ± 0.9a</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>2.5 ± 1.5a</td>
</tr>
<tr>
<td>SAA (µg/mL)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>4.8 ± 2.5a</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>6.1 ± 4.9a</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td></td>
</tr>
<tr>
<td>DIET</td>
<td>670 ± 110</td>
</tr>
<tr>
<td>DIET+EX</td>
<td>650 ± 71</td>
</tr>
</tbody>
</table>

Different superscript letters indicate significant difference between groups or time points for each variable (P < .05). Abbreviations: SAA, serum amyloid A; TNF-α, tumor necrosis factor-α.
concentrations relative to those in the DIET group. Ponies and Andalusians exhibited lower SI and higher AIRg values compared to Standardbreds, although each breed demonstrated the same trends across these variables over time.

A central finding of our study was the improvement in SI observed in animals that were exercised compared with those that were not. Previous studies have yielded conflicting results regarding the effect of exercise on glucose and insulin dynamics in equids, although heterogeneity exists among the characteristics of animals studied and the design of exercise programs evaluated. Low-intensity exercise without dietary modification appears insufficient to improve SI despite decreases in TBFM. A combined approach of dietary modification and low-intensity exercise yielded improvements in SI in obese ponies. Our study separated the effects of dietary restriction and exercise, providing evidence that a combined approach offers advantages over dietary restriction alone.

The reason for a lack of improvement in SI for the DIET group is unclear. This finding does not agree with several previous studies that achieved weight loss and improvements in SI or glucose tolerance by dietary restriction without prescriptive exercise. Direct comparisons among studies can be difficult because of different methodologies. For example, a study of obese Shetland ponies demonstrated improved glucose tolerance using a protocol that gradually restricted forage intake to as little as 35% of maintenance energy requirements, yielding much greater reductions in body mass of approximately 16% BW over 17 weeks. Greater levels of dietary restriction or a longer study period might therefore have yielded different results for animals in the DIET group.

The weight loss program in our study was designed to reflect current dietary recommendations for the management of animals with EMS. The restriction of forage to achieve weight loss while avoiding the potentially negative health consequences of negative energy balance, such as hyperlipemia, is suggested at 1.0%-1.5% BW. In addition to the restriction of DM intake, NSC content is a key consideration to minimize postprandial hyperinsulinemia, with existing recommendations suggesting an upper limit of 10% NSC content. Consistent with several previous studies of weight loss in equids, we chose to restrict forage to 1.25% BW (DM basis) using a mixed grass hay containing 9.1% NSC. A steady rate of weight loss similar to those of previous studies was observed without any untoward health consequences being appreciated. Feeding 4 times each day (and using slow feeders) aimed to ensure that although animals were without feed for 6 hours during daily turnout, periods without feed were no longer than would have been the case for previously described weight loss protocols.

Plasma adiponectin concentrations were higher in both DIET and DIET+EX groups after weight loss compared to starting concentrations. Adiponectin is an adipokine secreted by mature adipocytes that has insulin sensitizing and anti-inflammatory properties. Hypoadiponectinemia has been associated with insulin resistance in humans with metabolic syndrome, and also has been identified in equids with insulin resistance and a history of laminitis. Importantly, low plasma adiponectin concentration has been identified as a risk factor for future episodes of laminitis in ponies. Strategies that can ameliorate hypoadiponectinemia are therefore desirable in the management of animals with EMS to decrease the likelihood of laminitis. A previous study failed to detect changes in adiponectin in ponies after short-term low-intensity exercise without dietary modification. Our study supports the observation that a decrease in body fat, regardless of whether exercise was undertaken or not, might be important to improve plasma adiponectin concentration in equids.

Serum amyloid A concentrations were decreased in the DIET+EX group, but unchanged in the DIET group, after weight loss occurred. This finding is supportive of a beneficial effect of exercise in decreasing this biomarker of systemic inflammation, as previously demonstrated in ponies subjected to short-term low-intensity exercise without dietary modification. Conflicting evidence exists that EMS represents a chronic low-grade proinflammatory state. Serum amyloid A has been proposed as a useful biomarker of systemic inflammation in obese horses with insulin dysregulation. Although significant differences were detected in the DIET+EX group, the mean concentrations of SAA for both groups were within the reference interval for healthy horses.

Our study evaluated 3 different breeds: Standardbred horses, mixed-breed ponies, and Andalusian horses. Previous investigations by our group have reported innate differences in glucose and insulin dynamics among these breeds, with ponies and Andalusians having lower SI and higher AIRg values, as well as greater incretin responses to cereal grains, compared with Standardbreds. The present study affirmed these observations during a period of weight loss, with the same effect of breed observed for SI and AIRg values. There were no statistical interactions involving breed for FSIGT results or plasma measurements, and importantly, there were no differences in BCS or TBFM detected among breed groups, meaning that the observed breed differences in SI and AIRg were independent of relative adiposity.

Further evaluation of the weight loss program described here is warranted to determine whether our findings are broadly applicable. The animals enrolled had been obese for only a short period of time after a prior study of diet-induced weight gain, and exhibited a range of starting SI values. Whether the weight loss protocol described would have yielded different results using animals with chronic obesity or more severe insulin dysregulation is not known. We did not control for free activity during the period of daily turnout and did not quantify free activity during these times. However, the aim was to evaluate the effect of additional prescriptive low-intensity exercise as part of a practical weight loss program, and a period of daily turnout was considered important to the welfare of the animals studied.

Dietary modification to decrease caloric intake and minimize postprandial hyperinsulinemia remains the cornerstone of management for obese animals with EMS. In our study, weight loss was associated with decreases in basal insulin and leptin concentrations, and increases in adiponectin concentration, regardless of exercise status. The inclusion of regular low-intensity exercise resulted in improvements in SI and decreases in SAA. Although animals in our study were not selected with the prerequisite of severe insulin dysregulation, our findings were consistent across several breeds and a wide range of insulin sensitivities, supporting recommendations that exercise should be included, whenever possible, as part of a holistic management program for animals with obesity or EMS.
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CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
The use of animals in this study was approved by the University of Melbourne Animal Ethics Committee (ID: 1011918).

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

ORCID
Nicholas J. Bamford https://orcid.org/0000-0001-7675-9126

REFERENCES

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Author/s:
Bamford, NJ; Potter, SJ; Baskerville, CL; Harris, PA; Bailey, SR

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