

**STANDARD ARTICLE**

# Correlation between L-lactate and glucose concentrations and body condition score in healthy horses and ponies

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**Abstract**

**Background:** Blood L-lactate and glucose concentrations were higher in ponies with gastrointestinal disease than in horses, possibly because of differences in body condition (BC).

**Objectives:** To investigate whether L-lactate and glucose concentrations correlate with BC and differ between healthy horses and ponies.

**Animals:** Systemically healthy client-owned ponies (n = 101) and horses (n = 51).

**Methods:** Prospective observational study. Breed, weight, height, and subjective and objective measures of BC were recorded and L-lactate and glucose concentrations were measured. Correlations between L-lactate and glucose concentrations and BC were established. The association between L-lactate concentrations, equid type (pony or horse), BC, age, and glucose concentrations was investigated using a multivariable model.

**Results:** Weak but significant ( $P = .001$ ) negative correlations were detected between L-lactate concentration and average BC score ( $r = -0.29$ ), heart girth:height ratio ( $r = -0.27$ ), and age ( $r = -0.27$ ). Glucose concentrations were significantly ( $P < .001$ ) positively correlated with neck length:heart girth ratio ( $r = 0.37$ ) and heart girth:height ratio ( $r = 0.31$ ). L-lactate and glucose concentrations were weakly correlated ( $r = 0.15$ ;  $P = .04$ ). In the final multivariable model, age ( $-0.02 \pm 0.006$ ;  $P = .001$ ) and heart girth:height ratio ( $-1.74 \pm 0.53$ ;  $P = .001$ ) were significantly associated with the natural logarithm of L-lactate concentration ( $\text{LnL-Lactate}$ ). This represents a 2% decrease in L-lactate concentration per year increase in age and 10% decrease in L-lactate concentration per 0.06 unit increase in heart girth:height ratio.

**Conclusions and Clinical Importance:** In healthy horses and ponies, age and BC significantly influence L-lactate concentrations.

**KEYWORDS**

adiposity, body condition, equine, lactate metabolism

**1 | INTRODUCTION**

In recent studies investigating ponies and horses with gastrointestinal disease, blood L-lactate and glucose concentrations on admission were higher in ponies compared to horses regardless of the primary

**Abbreviations:** BC, body condition; BCS, body condition score; LnLact, natural logarithm of L-Lactate concentration; OGTT, oral glucose tolerance test.

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lesion.<sup>1,2</sup> The investigators speculated that ponies could have higher resting L-lactate concentrations either as a result of differences in body condition (BC), particularly obesity, or underlying metabolic differences such as a comparatively lower insulin sensitivity in ponies.

In people, higher blood L-lactate concentrations have been documented with obesity and type 2 diabetes, and increased L-lactate concentrations have been shown to predict the occurrence of diabetes in the future.<sup>3-7</sup> Increased glycolysis as a consequence of hyperinsulinemia in people with diabetes and insulin resistance leads to increased nicotinamide adenine dinucleotide hydrogen and pyruvate production. Conversion of pyruvate to L-lactate by lactate dehydrogenase appears to be exaggerated in insulin resistance secondary to the increased glycolysis. Lactate concentrations, therefore, reflect the state of cellular metabolism, and increased concentrations are thought to be an early signal of emerging insulin resistance.<sup>3</sup> Ponies, particularly obese ponies, are less sensitive to insulin as compared to large breed horses,<sup>8-10</sup> which could be a reason for the observed higher L-lactate concentrations in ponies with gastrointestinal disease.<sup>1,2</sup> Administration of exogenous corticosteroids to healthy horses decreases insulin sensitivity and increases L-lactate concentrations,<sup>11,12</sup> and increased free cortisol concentrations have been identified in overweight and obese equids.<sup>13</sup> It therefore is conceivable that differences in equid type (horse or pony) and measures of BC could influence blood L-lactate concentrations in otherwise healthy equids.

Our aim was to test the hypothesis that L-lactate and glucose concentrations are positively correlated with measures of BC and differ between healthy horses and ponies.

## 2 | MATERIALS AND METHODS

The Clinical Research Ethics Board of the Royal Veterinary College granted ethical approval for our study. Blood samples from ponies were obtained in the fall as part of an ongoing investigation (Home Office Project License [70/8195]) into predicting factors for future development of laminitis in healthy not previously laminitic ponies). Blood samples also were obtained from systemically healthy large breed horses presented to the Royal Veterinary College Equine Referral Hospital that had blood samples taken for reasons not relating to the study and were not receiving medications at the time of blood sampling. Written owner's consent was obtained for all veterinary procedures and for use of residual samples for research purposes. Animals were judged to be systemically healthy based on history and physical examination findings. Ponies and horses were defined by height: animals were considered a pony if the height was  $\leq 148$  cm (14.2 hands), whereas horses were defined as animals  $>148$  cm in height. All horses and ponies were weighed, and height, neck length, neck circumference, body length, heart, and belly girth were measured, and a cresty neck score was assigned as previously described.<sup>14</sup> Body condition scores (BCS) were assigned for 6 different body regions (neck, withers, shoulder, ribs, loin, and tail head) on a 1-9 scale as previously described,<sup>15</sup> and the average subjective BCS also was calculated. Blood samples were collected by jugular venipuncture into sample tubes containing sodium heparin,

potassium ethylenediaminetetraacetic acid, or sodium fluoride/potassium oxalate as anticoagulants. L-lactate concentrations were measured immediately after blood collection in heparinized samples using the handheld point of care analyzer NovaVet Statstrip Xpress Lactate meter (Nova Biomedical, Waltham, Massachusetts). The analyzer is manufactured for the European market by the same company that produces the Lactate Plus lactate meter and uses the same technology. The Lactate Plus lactate meter previously has been validated in horses.<sup>16</sup> In ponies, plasma was chilled immediately to 4-10°C and separated by centrifugation (2000g), separated into 1 mL aliquots and then stored at -80°C until analysis. Glucose measurements were performed on stored oxalate/fluoride plasma using a standard laboratory analyzer (Beckman Coulter AU680 Chemistry analyzer; High Wycombe, UK). In horses, blood glucose concentrations were measured in oxalate fluoride plasma immediately after collection as previously described.<sup>17</sup> Plasma was separated by centrifugation (1350g), and glucose was measured using a handheld glucometer Accu Chek Aviva (Roche Diagnostics Limited, Burgess Hill, West Sussex, UK) previously validated in horses and shown to have excellent agreement with laboratory standards.<sup>17</sup> Animals  $<1$  year of age were excluded from the study.

### 2.1 | Statistical analysis

Data were analyzed using a commercially available software program (SPSS version 24; SPSS Inc, Chicago, Illinois). Normality of the data was assessed using the Shapiro-Wilk test. Continuous data were expressed as mean  $\pm$  SD or median and range (minimum to maximum), and categorical data were presented as numbers and percentages. Comparison between equid types (horse versus pony) was done by 2-sample t-test (normally distributed continuous variables), Mann-Whitney U test (nonnormally distributed continuous variables), or chi-squared test (categorical data).

The average subjective BCS was calculated from 6 subjective measures of BC (BCS of neck, withers, shoulder, ribs, loin, and tail head). Objective measurements of BC (neck length:heart girth; neck circumference:height, and heart girth:height ratios) also were calculated. The correlations between L-lactate concentration and age, glucose concentration, average subjective BCS, and objective ratios of BC were evaluated using bivariate Spearman's correlation ( $r$ ). Correlations between glucose and the same variables mentioned above also were performed.

A linear model was employed to evaluate the association between L-lactate concentration and equid types, glucose concentration, and BC. Natural logarithm transformation was applied to L-lactate concentrations ( $\ln_{\text{lact}}$ ) before the analysis because values were not normally distributed. Variables with a  $P$  value  $<.1$  in the univariable analysis were assessed in the multivariable analysis, and a manual backward elimination procedure was adopted to obtain the final multivariable model. Results of the final linear models were displayed as regression coefficient  $\pm$  standard error. Because the response was  $\ln$  transformed, its expected change in the original scale and 95% confidence interval also was presented. Statistical significance was set at  $P \leq .05$ .

**TABLE 1** Comparison between sex, age, body condition parameters, and glucose and lactate concentrations between equid types

	n	Horse		Pony		P-value
		Male	Female	Male	Female	
Sex	152	32	19	68	33	.6
Age (years)	139	10.5 (1.5-24)		11 (5-27)		.24
Average body condition score	140	5.2 ± 1.3		5.8 ± 1.0		.001
Neck length:heart girth (cm)	139	0.51 (0.43-0.61)		0.32 (0.25-0.37)		<.001
Neck circumference: height (cm)	139	0.64 (0.55-0.79)		0.62 (0.48-0.77)		.061
Heart girth:height (cm)	139	1.19 ± 0.06		1.25 ± 0.07		<.001
L-lactate concentration (mmol/L)	152	0.9 (0.2-1.9)		0.7 (0.2-2.7)		.05
Glucose concentration (mmol/L)	152	5.2 (3.7-7.2)		4.6 (3.9-5.7)		<.001

A 2-sample *t*-test was used for normally distributed variables (average subjective body condition score and heart girth to height ratio); a chi-squared test was used for categorical data (sex) and a Mann-Whitney *U* test was used for nonnormally distributed continuous data (remaining variables).

**TABLE 2** Correlation between L-lactate and glucose concentrations and age and measures of body condition in horses and ponies (n = 152)

L-Lactate concentration (horses and ponies, n = 152)	Spearman's rho	P-value	Glucose	Spearman's rho	P-value
Average body condition score	-0.29	.001		-0.016	.85
Neck length: heart girth	0.16	.07		0.37	<.001
Neck circumference: height	-0.15	.09		0.01	.87
Heart girth: height	-0.27	.001		0.31	<.001
Age	-0.27	.001		-0.01	.88
Glucose concentration	0.15	.04	L-Lactate	0.15	.04

Statistical significance was set at *P*-value of ≤.05.

### 3 | RESULTS

One hundred one ponies and 51 horses comprising 100 geldings and 52 mares with a median age of 11 years (range, 1.5-27 years) were included in the study. L-lactate and glucose concentrations and several measures of BC differed significantly between horses and ponies (Table 1). Results of correlations between L-lactate concentration, average subjective BCS, age, and glucose concentrations are displayed in Table 2. Correlations between the same variables and glucose concentration also are displayed in Table 2.

Factors included in the multivariable model (*P* < .1) were age, glucose concentration, average subjective BCS, and heart girth:height. Factors not included were sex (*P* = .85), equid type (*P* = .15), neck length:girth ratio (*P* = .12), and neck circumference:height ratio (*P* = .17). In the final multivariable model, age (-0.02 ± 0.006; *P* = .001) and heart girth:height ratio (-1.74 ± 0.53; *P* = .001) were significantly associated with L-lactate in log scale. This represents a 2% (1 - exp[-0.02]) decrease in L-lactate concentration per 1 year increase in age and 10% (1 - exp[-1.74 × 0.06]) decrease in L-lactate concentration per 0.06 unit increase in heart girth:height ratio.

### 4 | DISCUSSION

Similar to previous studies,<sup>14</sup> ponies compared to horses had higher average BCS and higher heart girth:height and lower neck length:heart

girth ratios, indicating that adiposity was more widespread in ponies. Contrary to expectations and in contrast to previous studies in horses and ponies with gastrointestinal disease,<sup>1,2</sup> healthy ponies had significantly lower blood L-lactate concentrations compared to horses. L-lactate concentrations were weakly, but significantly, associated with measures of adiposity. Also contrary to expectations, the correlation was negative, indicating that leaner animals had higher L-lactate concentrations. Thus, because equid type was not significantly associated with L-lactate in the multivariable model, the differences between healthy horses and ponies in our study likely can be attributed to the differences in BC. In people, L-lactate is released from both adipose tissue and skeletal muscle, and in healthy subjects release from muscle exceeds that from adipose tissue.<sup>18</sup> However, it is likely too simplistic to attempt to explain higher L-lactate concentrations solely by higher muscle mass in leaner equids. Circulating L-lactate concentrations reflects the sum of L-lactate release and uptake from various tissues. L-lactate dynamics vary widely among different tissues and during postprandial states versus hyperinsulinemic states. To complicate matters, there are not only differences between lean and obese individuals but insulin sensitivity and fat distribution in the body also play a role.<sup>18,19</sup> More research is needed in the area before the association between L-lactate and BC in equids can be explained.

It previously had been hypothesized that higher L-lactate concentrations observed in ponies with gastrointestinal disease possibly could be explained by higher resting L-Lactate concentrations in healthy ponies,

possibly as a result of lower insulin sensitivity, higher degree of adiposity, or both. Although insulin sensitivity was not determined in our study, previous studies have identified decreased insulin sensitivity in overweight and obese animals.<sup>8</sup> Because measures of adiposity were negatively correlated with L-lactate concentrations, a clinically relevant role of insulin sensitivity on L-lactate concentrations seems less likely. However, this conclusion should be confirmed by further investigations. Differences in glucose and insulin metabolism among equine breeds frequently are not noticeable in a resting state but only become apparent after dynamic response testing.<sup>8,10</sup> An oral glucose tolerance test (OGTT) performed in healthy human volunteers not only showed higher baseline L-lactate concentrations in participants with an OGTT result suggestive of insulin resistance but also showed progressively increasing L-lactate concentrations throughout the test whereas concentrations remained stable in volunteers with a normal test result.<sup>3</sup> It is possible that L-lactate metabolism in ponies responds differently when exposed to stressors such as pain, hypovolemia, catecholamine release, and systemic inflammation with concentrations increasing more rapidly or to a greater extent. In people, physical exercise provokes a stress response. Normal stress in the form of regular exercise leads to an abatement of the hormonal stress response that also can be apparent when the athlete is exposed to other life stressors.<sup>20</sup> Fitness and exercise therefore could also play a role, possibly in multiple ways by affecting body composition, insulin sensitivity, and stress responses.<sup>20-23</sup>

In our study, age was significantly negatively correlated with L-lactate concentrations. The association between age and L-lactate concentrations has been described previously in foals in the immediate postpartum period with initially increased concentrations decreasing to within the adult reference range within 3-5 days after birth,<sup>24,25</sup> whereas in horses and ponies with gastrointestinal disease, age was not significantly associated with L-lactate concentrations in a multivariable model.<sup>2</sup> A negative correlation between L-lactate and age also has been observed in people at rest, but the investigators offered no explanation for this finding.<sup>26</sup> Progressively lower L-lactate concentrations during exercise in older people have been attributed to declining anaerobic energy production from glycolysis with advancing age.<sup>27,28</sup> Whether this could also be the case in equids needs to be determined.

As shown previously in equids with gastrointestinal disease and in horses and ponies with atypical myopathy, L-lactate and glucose concentrations were positively correlated.<sup>2</sup> In contrast to diseased animals, the correlation in our study was weak, indicating that in healthy animals under resting conditions, glucose concentrations only have a very minor influence on L-lactate concentrations. Previous studies in healthy animals have shown either no differences in glucose concentrations<sup>10,14</sup> or, similar to our findings, lower glucose concentrations in healthy ponies compared to horses.<sup>29</sup> This is in contrast to studies in diseased ponies in which glucose concentrations were higher compared to horses.<sup>2</sup> Because glucose concentrations were positively correlated with measures of adiposity, which was more pronounced in ponies, higher concentrations in ponies might have been expected. Although a different method was used to determine glucose concentrations in horses, good agreement has been demonstrated between the methods.<sup>17</sup> In a previous study, the handheld device used in our

study consistently measured lower concentrations compared to the laboratory method,<sup>17</sup> suggesting that the actual difference might have been underestimated rather than overestimated.

Our study had several limitations including the relatively small number of animals investigated and the unequal number of horses and ponies. This was a consequence of financial limitations and the practical difficulties of recruiting suitable systemically healthy horses in a hospital population. Ideally, reference techniques would have been used for all lactate measurements rather than handheld devices, which could have increased the accuracy of the measurements. Use of the same analysis method for glucose measurements also would have been preferable. However, all methods used in our study had been validated in horses previously, and good agreement between methods had been demonstrated, hopefully limiting any negative impact on the study.

In conclusion, L-lactate concentrations in healthy equids are predominantly influenced by age and BC, whereas type (horse versus pony) seemed to play a minor, if any, role. The differences in L-lactate metabolism between healthy and diseased subjects require further investigation, particularly determining the influence of adrenergic stimulation on L-Lactate concentrations.

#### 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 Clinical Research Ethics Board of the Royal Veterinary College granted ethical approval for this study. Blood samples from ponies were obtained in the fall as part of an ongoing investigation (Home Office Project License (70/8195).

#### HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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**How to cite this article:** Dunkel B, Knowles EJ, Chang Y-M, Menzies-Gow NJ. Correlation between L-lactate and glucose concentrations and body condition score in healthy horses and ponies. *J Vet Intern Med*. 2019;33:2267-2271. <https://doi.org/10.1111/jvim.15587>