

# Cardiac output determination by use of lithium dilution during exercise in horses

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**Objective**—To compare cardiac output (CO) obtained by the lithium dilution method (LiDCO) with CO calculated from the Fick principle (FickCO), in horses maximally exercising on a high-speed treadmill.

**Animals**—13 Thoroughbreds.

**Procedures**—In part 1 of the study, 5 horses performed a warm-up (walk, trot, and canter) and exercise test (walk, trot, canter, and gallop [90% to 100% maximum oxygen consumption [ $\dot{V}O_{2max}$ ]]) with measurements of LiDCO and FickCO obtained simultaneously after 60 seconds at each exercise level, for a total of 7 measurements. In part 2 of the study, 8 horses performed a warm-up (walk, trot, and canter) followed by an exercise test (walk and gallop [90% to 100%  $\dot{V}O_{2max}$ ], repeated twice). Measurements of LiDCO and FickCO were obtained 60 seconds into the first walk and each gallop of the exercise tests, for a total of 3 measurements.

**Results**—Cardiac output increased significantly with increasing speeds by use of both methods. In part 1, lithium dilution significantly overestimated CO, compared with the Fick principle, during the exercise test (as both injection number and exercise intensity increased). Mean  $\pm$  SD bias was  $246 \pm 264$  mL of blood/min/kg in part 1 and  $67 \pm 100$  mL of blood/kg/min in part 2. Three injections of lithium (part 2) did not result in the same degree of overestimation of LiDCO that was observed with 7 injections (part 1).

**Conclusions and Clinical Relevance**—Lithium dilution may be an acceptable substitute for the Fick principle as a means to measure CO in maximally exercising client-owned horses. (*Am J Vet Res* 2008;69:1054–1060)

Cardiac output provides a useful measurement of cardiovascular function and the capacity for whole-body oxygen delivery. In human medicine, this information is used extensively during anesthesia<sup>1</sup> and to monitor critically ill patients.<sup>2</sup> Recent advances in techniques are making similar information available to veterinary clinicians.<sup>3–5</sup> Cardiac output measurements are also useful in describing normal physiologic responses to exercise. In horses, measurement of CO could be extremely useful in the diagnosis of potential performance-limiting cardiac abnormalities that may only manifest during exercise. A number of methods exist to measure CO in horses.<sup>6</sup> These include the indicator dilution methods (ie, thermodilution, indocya-

ABBREVIATIONS	
CO	Cardiac output
CV	Coefficient of variation
FickCO	Cardiac output calculated from the Fick principle
LiDCO	Cardiac output determined by the lithium dilution method
LOA	Limits of agreement
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximum oxygen consumption

nine green, and direct impedance), echocardiography, and the direct or indirect Fick principle.<sup>7</sup> With the exception of the direct Fick principle, these techniques have been found to be unsuitable in maximally exercising horses. Although the Fick principle provides an accurate, reproducible measurement of CO in horses during exercise, technical requirements make it unsuitable for most uses in a clinical setting. Requirements for a system to measure  $\dot{V}O_2$  (a facemask, the capability of measuring expired oxygen concentrations, and, most importantly, a tractable horse), combined with the need for invasive placement of a catheter in the pulmonary artery, prevent its use in most situations other than research settings.

The development of the lithium dilution method<sup>8</sup> may provide a possible alternative to the Fick principle

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to measure CO in exercising horses. The lithium dilution method is a form of indicator dilution in which lithium chloride is used as the injectate. In this method, a known volume and concentration of lithium is rapidly injected into a vein and the lithium concentration is measured from a peripheral artery by a sensor. The LiDCO computer generates a lithium concentration-time curve, and CO is calculated from the area under the curve by use of the Stewart-Hamilton equation.<sup>9,10</sup> Thus, the dilution of the lithium is directly proportional to the total amount of blood passing through the heart (ie, CO). The advantage of this technique over other indicator dilution techniques is its reduced invasiveness. It requires catheterization of only a peripheral vein<sup>11,12</sup> and artery and avoids the need to catheterize the pulmonary artery. The suitability of the lithium dilution method to measure CO in human medicine has been reported,<sup>13,14</sup> and recently, comparison with other methods in clinical veterinary patients has also been reported.<sup>3-5,15</sup> However, the use of LiDCO during high-intensity exercise in horses has yet to be investigated. Therefore, the purpose of the study reported here was to compare CO measurements obtained with the commonly used Fick principle with those obtained by use of the newer lithium dilution method in horses exercising up to maximal intensities.

## Materials and Methods

**Animals**—Thirteen Thoroughbreds (2 females and 11 males) in good health, ranging in age from 3 to 6 years old and weighing from 470 to 550 kg, were trained to run on a high-speed treadmill<sup>a</sup> and conditioned for  $\geq 8$  weeks before studies were started. Horses lived in stalls, were fed grain (1.5 kg/horse; 14% protein) twice daily, and received ad libitum hay and water. Anthelmintics and vaccinations were administered as appropriate for the region.

After the conditioning period, an incremental exercise step test was conducted with each horse to determine running speeds necessary to elicit  $\dot{V}O_2$  max as previously described.<sup>16-18</sup> All protocols and procedures involving horses for this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

**Experimental protocol**—This study was conducted in 2 parts. Part 1 was designed to evaluate multiple injections of lithium to determine CO over a range of gaits, compared with CO measurements obtained simultaneously by use of the Fick principle. This study also compared the FickCO obtained during lithium injection with FickCO obtained during saline (0.9% NaCl) solution injection to determine whether injection of lithium had a large influence on FickCO. Part 2 was then performed in an effort to separate the effect of speed from the effect of injection number because of a consistently increasing overestimation of LiDCO as both the number of injections and the exercise intensity increased. During part 2, the total number of lithium injections was decreased to evaluate the highest speed at the time of the second injection. This was done to avoid excess background accumulation of lithium, which may cause an overestimation of CO.

## PART 1

Horses participated in 2 exercise trials randomly assigned and separated by 10 to 14 days. Instrumentation (description to follow) and exercise were identical for the 2 trials. Each trial consisted of a warm-up period of 2 minutes each at a walk (1.5 m/s), trot (4.0 m/s), and canter (8.0 m/s), followed by 2 minutes each at a walk (1.5 m/s), trot (4.0 m/s), canter (8.0 m/s), and gallop. The gallop was performed at 90% to 100% of the speed sufficient to elicit  $\dot{V}O_2$  max (13 to 15 m/s; 0° incline). For the first trial, lithium chloride<sup>b</sup> (16.5 mL; 600mM solution [ie, 9.9 mmol/horse or approx 0.8 to 0.9 mg/kg]) was rapidly injected into a jugular vein after 60 seconds at each exercise intensity, including the warm-up period, for a total of 7 injections to determine LiDCO. This was compared with simultaneously derived FickCO. For the second trial, an equal volume of saline solution was substituted for the lithium chloride injection during determination of FickCO to determine whether there was an effect of lithium chloride injection on FickCO. Although comparisons of FickCO during saline solution injection and FickCO during lithium injection could not be conducted simultaneously and are therefore indirect comparisons, the same horse at the same fitness level working at the same exercise intensity (ie, approx 100% of the speed necessary to elicit  $\dot{V}O_2$  max) is reported to have a CV of  $\leq 12.5\%$  between repetitive measurements of CO.<sup>18</sup>

## PART 2

Horses participating in part 2 of the study were instrumented as for part 1 (description to follow). The same volume and concentration of lithium chloride as for part 1 was injected after 60 seconds at the appropriate exercise intensity. In part 2, horses were warmed up prior to lithium chloride injections by use of the same exercise protocol as for part 1. The warm-up was followed by a 2-minute walk and a 2-minute gallop at 90% to 100% of the speed previously determined to elicit  $\dot{V}O_2$  max (13 to 15 m/s). This sequence was repeated, and horses walked after the final gallop. Lithium chloride was injected during the first walk and during each of the 2 gallops, for a total of 3 injections. The value of FickCO was determined at the time of LiDCO for comparison.

**Instrumentation and sample collection**—In part 1, following aseptic preparation of the sites and local infiltration with 2% lidocaine, a 20-gauge catheter was placed into a transverse facial artery, a 14-gauge catheter and an 8.5-F catheter introducer were placed into the left jugular vein, and an 18-gauge catheter was placed into the right carotid artery that had previously been surgically elevated to a subcutaneous position. A check-valve with side-port was attached to the 14-gauge jugular catheter to permit passage of a thermocouple temperature probe<sup>c</sup> to the level of the right atrium and rapid injection of lithium chloride or saline solution into the vein. A catheter was passed through the 8.5-F introducer to the level of the pulmonary artery. Correct placement was verified by following characteristic pressure waveform changes as the catheter was advanced. Horses in part 2 were instrumented in the same manner, except they did not have a catheter placed in the right carotid artery.

Oxygen consumption and carbon dioxide production were measured continuously with an open-circuit system as previously described.<sup>16,18-20</sup> A data acquisition system<sup>d</sup> was used to convert analog signals from the gas analyzer,<sup>e</sup> treadmill, ECG monitor,<sup>f</sup> and flowmeter<sup>g</sup> to digital signals.

In part 1, blood samples were drawn anaerobically into heparinized syringes simultaneously from the pulmonary artery and carotid artery after lithium chloride or saline solution injection for determination of FickCO and correction of LiDCO. Samples were stored on ice until analysis, within 15 minutes, for measurement of total hemoglobin,<sup>h</sup> blood lactate and glucose concentrations, blood gas tensions, and plasma electrolyte (Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>) concentrations. Oxygen content was calculated from the hemoglobin concentration by use of a hemoximeter.<sup>h</sup> Blood lactate and glucose concentrations were assayed by an automated lactate analyzer,<sup>i</sup> and blood gas tensions and plasma electrolyte concentrations were measured by use of a clinical blood gas system.<sup>j</sup> Heart rate and rhythm were recorded continuously throughout the exercise test.

In part 2, blood samples were drawn anaerobically through the pulmonary artery and transverse facial artery catheters after lithium chloride injection, as soon as an area under the curve was computed for determination of FickCO and correction of LiDCO. The sample storage and analysis were the same as for part 1.

**Measurement of CO**—A commercial LiDCO cardiac computer system<sup>k</sup> was used to compute LiDCO from the lithium concentration measured by a lithium sensor. The sensor was connected to the transverse facial artery catheter via an extension set and 3-way stopcock and to a peristaltic pump with a 3-way stopcock and tubing. The pump was used to withdraw blood from the artery at a constant rate of 40 mL/min across the sensor. Prior to studies, blood from the sensor was collected over timed periods to determine flow rate and confirm constant speed of the pump. The LiDCO cardiac computer software<sup>k</sup> adjusted calculated CO for plasma Na<sup>+</sup> and hemoglobin concentrations. Because concentrations of these 2 blood components can change substantially during exercise in horses, baseline concentrations were initially entered into the computer. The actual plasma concentrations of Na<sup>+</sup> and hemoglobin, determined as described, were later used to adjust CO for any deviations from initial concentrations.

The Fick principle<sup>7</sup> was used to calculate CO from simultaneously measured  $\dot{V}O_2$  and oxygen content in arterial (CaO<sub>2</sub>) and pulmonary arterial (CvO<sub>2</sub>) blood according to the following equation:

$$CO = \frac{\dot{V}O_2}{CaO_2 - CvO_2}$$

For LiDCO and FickCO, sample collection occurred 60 seconds into exercise to achieve steady-state conditions at each of the indicated intensities.

**Data analysis**—The relationship between CO measurements derived by use of the 2 methods was explored with clustered regression analysis. For this analysis, all data for the lithium dilution method were

regressed on the CO values obtained by use of the Fick principle for each part of the study. To accommodate the use of multiple observations per horse in the analysis, clustered regression was used. Clustered regression is a regression method that maintains rigor and robustness when repeated subject-level observations exist in a regression dataset.<sup>21</sup> In part 1, data were clustered into 7 groups representing individual exercise intensities. Similarly, data from part 2 were clustered into 3 separate groups.

Data were also analyzed by use of the Lin concordance correlation, which compares 2 techniques measuring the same variable without the inherent bias of establishing a gold standard.<sup>22,23</sup> The concordance correlation coefficient ( $\rho$ ) indicates agreement between the 2 measurements, with a value of 1 indicating perfect concordance. Analysis by use of the Lin concordance correlation<sup>22,23</sup> does not accommodate clustering considerations, and consequently, a refined bootstrapping approach was used for this aspect of the analysis. Three distinct considerations were included to maintain the integrity and robustness of the analysis. First, to avoid duplicating horses within each separate concordance determination, only single replications of horses were allowed. Second, to remove bias associated with selection of exercise intensity of the horse, in each of the concordance estimations, random characterizations (exercise intensity) of each horse were imposed. Finally, to derive representative concordance estimates for each of the 2 parts of the study, replicated runs of concordance estimates were introduced. This bootstrapping approach was run 100 times for each of the 2 parts of the study, and data from the resultant concordance postprocessing file were averaged to yield our estimates of the final concordance correlation coefficients and their variabilities. One hundred runs were required to produce consistent estimates of the concordance within each of the 2 study combinations.

The bias (mean difference between the 2 methods) and LOA of the 2 test methods were analyzed by use of the method described by Bland and Altman,<sup>24,25</sup> recently modified for use with multiple observations per individual.<sup>26</sup> The bias represents the systematic departure between the 2 measurement methods. The upper and lower LOA were calculated as bias  $\pm$  2 times the SD and define the range in which 95% of the differences between the 2 techniques lie. In addition, the bias and LOA for the FickCO obtained during lithium chloride injection and the FickCO obtained during saline solution injection were analyzed by use of the Bland-Altman method.<sup>26</sup>

Normality of the clustered data from each of the 2 parts of the study was ascertained with the Shapiro-Wilks test. Values of  $P \leq 0.05$  were considered significant. All analyses were performed with a commercially available statistical software package.<sup>1</sup>

## Results

**Part 1**—Cardiac output computed by the Fick principle and lithium dilution increased as exercise intensity increased. Mean  $\pm$  SEM FickCO was 297  $\pm$  25 mL of blood/kg/min at the initial walk and 692  $\pm$  24 mL of blood/kg/min at the gallop. The LiDCO was 381

$\pm 63$  mL of blood/kg/min at the initial walk and  $1,249 \pm 119$  mL of blood/kg/min at the gallop. The FickCO obtained during saline solution injection also increased significantly with exercise intensity ( $324 \pm 16$  mL of blood/kg/min at the initial walk and  $717 \pm 39$  mL of blood/kg/min at the gallop) and was not significantly different from FickCO obtained during lithium chloride injection.

Mean CV for FickCO over all speeds was 16.3% (range, 7.6% to 21.5%). Similar values were found for FickCO computed for the exercise trials when saline solution was injected (mean, 14.1%; range, 8.2% to 22.0%). However, for LiDCO, the mean CV was 29.6% (range, 20.2% to 37.1%).

Comparison of LiDCO with FickCO by use of the Bland-Altman method for multiple observations per individual<sup>26</sup> gave a mean  $\pm$  SD bias of  $246 \pm 264$  mL of blood/min/kg (Figure 1); LOA for the mean difference were  $-281$  to  $774$  mL of blood/min/kg. Similar analysis of FickCO measured during lithium chloride and saline

solution injections gave a bias of  $-22 \pm 74$  mL of blood/min/kg (data not shown); LOA for the mean difference were  $-171$  to  $126$  mL of blood/min/kg.

Mean concordance correlation coefficient (agreement,  $\rho \pm$  SD), as computed by use of the modifications of the Lin technique,<sup>22,23</sup> was poor for FickCO versus LiDCO ( $0.37 \pm 0.19$ ;  $P < 0.001$ ). Clustered regression analysis of FickCO versus LiDCO for part 1 of the study had a significant clustered regression coefficient (mean  $\pm$  SE,  $1.50 \pm 0.21$ ;  $P = 0.002$ ), whereas the intercept was not significant ( $P = 0.80$ ; Figure 2).

**Part 2**—Because of the increasing overestimation of LiDCO, compared with FickCO, as the number of injections and the exercise intensity increased in part 1, we attempted to separate the effect of increased CO at increased exercise intensities from the effect of number of injections on overestimation of CO. In part 2 of the study, CO was measured at the gallop after only 1 prior lithium chloride injection.

The CO for both measurement techniques (lithium dilution and Fick principle) increased with increasing

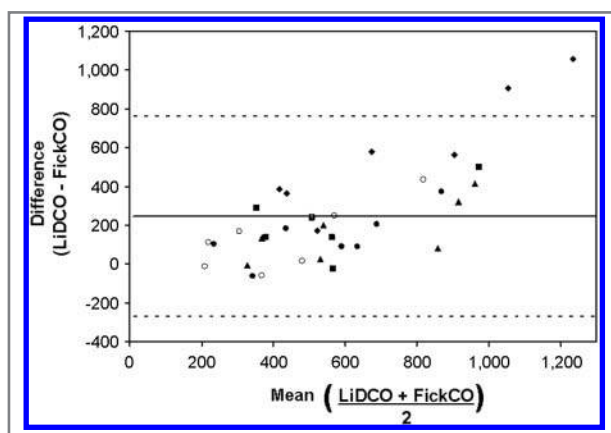


Figure 1—Bland-Altman plot of values for CO (mL of blood/min/kg) for data from 5 horses in part 1 of the study representing the relationship between the difference in FickCO and LiDCO and the mean of the 2 values. Each symbol represents a paired observation with different symbol types representing individual horses. 95% LOA for the mean difference =  $-281$  to  $774$  mL of blood/min/kg (dashed lines), with a mean  $\pm$  SD bias of  $246 \pm 264$  mL of blood/kg/min (solid line).

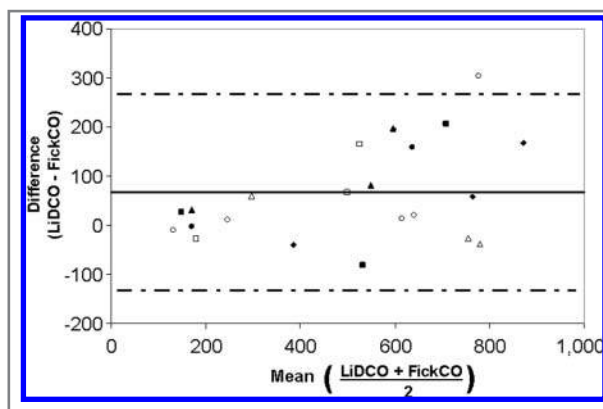


Figure 3—Bland-Altman plot of values for CO (mL of blood/min/kg) for data from 8 horses in part 2 of the study representing the relationship between the difference in FickCO and LiDCO and the mean of the 2 values. Each symbol represents a paired observation with different symbol types representing individual horses. 95% LOA for the mean difference =  $-132$  to  $265$  mL of blood/min/kg (dashed lines), with a mean  $\pm$  SD bias of  $67 \pm 100$  mL of blood/kg/min (solid line).

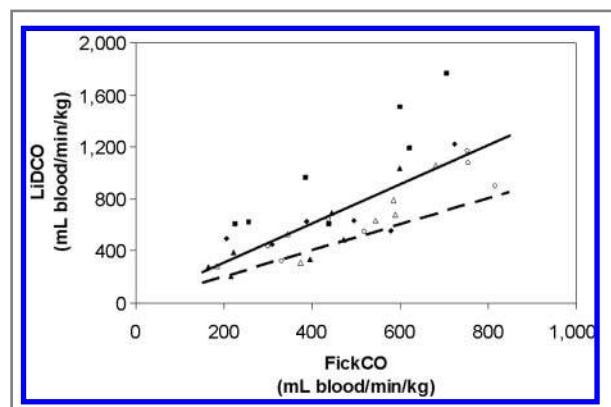


Figure 2—Relationship between FickCO and LiDCO for data collected from 5 horses in part 1 of the study. Different symbol types represent paired data from individual horses. The dashed line indicates perfect concordance, whereas the solid line provides the slope from the clustered regression analysis (slope = 1.50; intercept = 14.02;  $R^2 = 0.58$ ;  $P < 0.01$ ).

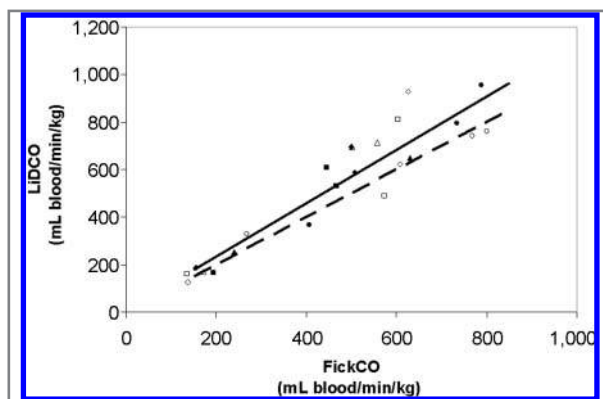


Figure 4—Relationship between FickCO and LiDCO for data collected from 8 horses in part 2 of the study. Different symbol types represent paired data from individual horses. The dashed line indicates perfect concordance, whereas the solid line provides the slope from the clustered regression analysis (slope = 1.12; intercept = 11.75;  $R^2 = 0.86$ ;  $P < 0.001$ ).

speed. Mean  $\pm$  SEM FickCO was  $213 \pm 30$  mL of blood/kg/min at the walk,  $601 \pm 39$  mL of blood/kg/min at the first gallop, and  $614 \pm 45$  mL of blood/kg/min at the second gallop. Mean  $\pm$  SEM LiDCO was  $219 \pm 29$  mL of blood/kg/min at the walk,  $655 \pm 35$  mL of blood/kg/min at the first gallop, and  $765 \pm 50$  mL of blood/kg/min at the second gallop.

Comparison of LiDCO with FickCO by use of the Bland-Altman method for multiple observations per individual<sup>26</sup> gave a mean  $\pm$  SD bias of  $30 \pm 79$  mL of blood/kg/min for the first walk and first gallop; LOA for the mean difference were  $-128$  to  $188$  mL of blood/min/kg. When all 3 measurements (walk, first gallop, and second gallop) were used to compare LiDCO with FickCO, a mean  $\pm$  SD bias of  $67 \pm 100$  mL of blood/kg/min was found (Figure 3); LOA for the mean difference were  $-132$  to  $265$  mL of blood/min/kg.

Mean concordance correlation coefficient (agreement,  $\rho \pm$  SD), as computed by use of the modifications of the Lin technique,<sup>22,23</sup> was moderately strong for FickCO versus LiDCO ( $0.84 \pm 0.12$ ;  $P < 0.001$ ). Clustered regression analysis of FickCO versus LiDCO for part 2 of the study had a significant clustered regression coefficient (mean  $\pm$  SE,  $1.12 \pm 0.10$ ;  $P < 0.001$ ), whereas the intercept was not significant ( $P = 0.76$ ; Figure 4).

## Discussion

This study compared simultaneous estimates of LiDCO and FickCO in exercising horses. The Fick principle has long been the most commonly used method of evaluating CO by researchers in exercising horses and is considered to be the most reliable method when exercise intensity is high. Although commonly used by researchers, the Fick principle of measuring CO is technically demanding, requires sophisticated equipment, requires extensive instrumentation, and because of the requirement of a facemask, is not routinely tolerated by client-owned horses. However, it is currently the best technique available to measure CO in maximally exercising horses. Other methods of measurement of CO have been unsuccessful in horses undergoing high-intensity exercise. Thermodilution, although accurate in standing or anesthetized horses, is not accurate at high speeds and CO. This is most likely caused by the rapidly changing core body temperatures of exercising horses and an inability to inject sufficient cold saline solution to yield a sufficient temperature difference following dilution at high flow rates. In addition, problems in bolus administration (ie, handling of the syringe and its effect on injectate temperature and rapidity of injection of relatively large volumes) may result in inaccurate measurements. Dye dilution techniques that use indocyanine green have also been unsuccessful. It is difficult to rapidly inject a sufficient quantity of dye to be able to detect changes in dye concentration above background at the high CO attained during intense exercise in horses. This is a concern related to all indicator dilution techniques, including the lithium dilution method. Additionally, in humans, there is a concern of adverse or allergic reactions to the dye.<sup>27</sup> The use of Doppler echocardiography for the measurement of CO has been investigated. Although a reasonable correlation between thermodilution and either trans-

esophageal or transthoracic echocardiography exists in anesthetized or standing horses,<sup>4,28,29</sup> these procedures cannot be done in exercising horses.

The recently described lithium dilution method has been used in humans and animals to measure CO. It was developed to avoid the requirement to catheterize the pulmonary artery and to provide a simpler, safe, reliable means to measure CO. Linton et al<sup>8</sup> evaluated its use in humans and found it to be accurate, compared with thermodilution. In swine, it was found to be as accurate as thermodilution, compared with electromagnetic flowmetry.<sup>13</sup> Linton et al<sup>4</sup> compared lithium dilution, thermodilution, and transesophageal Doppler echocardiography as a means to measure CO in anesthetized horses and found that lithium dilution was accurate, safe, and technically easy to perform, compared with the other techniques. It has also been used successfully to monitor CO in anesthetized<sup>15</sup> and sick<sup>30</sup> neonatal foals. Lithium dilution was also found to be accurate for the measurement of CO in dogs, compared with thermodilution, and to obviate the need for a pulmonary artery catheter.<sup>5</sup> It was concluded to be safe, reliable, and simple to use, compared with thermodilution, and relatively cost effective.

To determine whether lithium chloride injection affected FickCO, values of CO were compared when either saline solution or lithium chloride was simultaneously injected in a random order. A significant bias was not found between these 2 values for each speed, and the CO values were not significantly different from each other, suggesting that the lithium chloride injection itself did not greatly influence CO. Variation of FickCO in part 1 of the study was similar to that previously reported.<sup>18</sup> Variation of LiDCO in part 1 of the study was somewhat greater than previously published for dogs<sup>5</sup> and horses<sup>4,14</sup>; however, the present study was conducted during various exercise intensities and high CO, whereas the previous studies were conducted on anesthetized or resting animals.

During the second series of walk, trot, canter, and gallop, as the number of injections and exercise intensity increased, an increase in the mean bias was observed, indicating that lithium dilution consistently and significantly provided an overestimation of CO. From this initial study, the reason for the overestimation could not be determined with certainty, but findings in recent studies<sup>5,31</sup> indicate that this could be the result of several factors, including excessive buildup of background lithium, sensor overuse, or inherent inaccuracies of the lithium dilution method at high speeds.

Given the selectivity of the lithium sensor, there is a theoretic upper limit for background lithium concentration of  $0.2$  mmol/L to accurately measure CO with the lithium dilution system as provided.<sup>5</sup> High background concentrations of lithium could interfere with the ability of the sensor to distinguish the lithium chloride injection from background concentrations, resulting in a decrease in the measured concentration change and an overestimation of the true CO.<sup>31</sup> It was beyond the scope of our study to measure plasma lithium concentrations as a means to determine background concentrations; however, it is likely that the lithium background concentration exceeded this theoretic upper

limit in part 1. By use of the pharmacokinetic model of lithium distribution in nonexercising horses by Hatfield et al,<sup>32</sup> estimated plasma lithium concentration after 5 injections (following the second trot) would have been approximately 0.5 mmol/L.

In part 2 of the study, the use of fewer injections was undertaken to remove the potential influence of excessive background lithium concentrations and sensor wear on results. In part 2 of the study, the plasma lithium concentration after 1 injection would have been approximately 0.1 mmol/L<sup>32</sup>, thus, for the first gallop, excess background lithium should not have accumulated. The calculated estimation of plasma lithium concentration after 2 injections would have been approximately 0.2 mmol/L on the basis of the model presented by Hatfield et al.<sup>32</sup>

Results of the Bland-Altman analysis in part 2 of the measurements at the walk and the first gallop suggest good agreement, without overestimation of LiDCO. Analysis of all 3 measurements (walk, first gallop, and second gallop) gave a moderate correlation and had an increased mean bias, compared with the first 2 injections, suggesting that either a limited number of measurements can be made when the high concentrations of lithium necessary during high speed and high CO measurements are used or there is a decreased reproducibility of measurements at high speeds. However, both these analyses in part 2 had much better correlations than after 7 injections of lithium in part 1. Simply limiting the number of injections within a short period should prevent the excess lithium background accumulation and might improve accuracy.

The amount of lithium chloride to be injected was determined in a small pilot study. The amount was selected to reliably create a peak signal of  $\geq 0.25$  mV, well within the limits of detectability of the lithium sensor, during exercise at intensities sufficient to elicit  $\dot{V}O_{2\max}$ . Furthermore, the selected amount of lithium chloride injected (9.9 mmol) represents the maximum amount of lithium that the software<sup>k</sup> was configured to accept. A recent study<sup>32</sup> evaluated the potential toxicity of lithium chloride in horses. In this study, a total of 60 mmol of lithium chloride was injected as 20 separate 3-mmol boluses over 60 minutes. This was considered to be in excess of the total amount that would typically be administered to measure CO in resting or anesthetized horses over a 2- to 4-hour period. Plasma lithium concentrations as well as toxic effects were assessed, with no evidence of lithium toxicosis seen. The cumulative dose resulted in a mean peak plasma concentration of 0.5493 mmol/L, far below the reported toxic concentrations in other species (1.5 mmol/L in dogs<sup>33</sup> and 1.6 mmol/L in humans<sup>34</sup>). For part 1 of the study, a total of 70 mmol of lithium chloride was injected over a 15-minute time interval. None of the horses displayed any signs of potential adverse effects.

Another potential source of error in the lithium dilution method could be related to the rate at which the peristaltic pump drew blood through the sensor, combined with prolonged use of the same sensor. To reproducibly measure CO in these horses during exercise, it was necessary to pump blood through the sensor at a rate greater than the manufacturer originally intended.

During a pilot study, increasing pump speeds were tried until the primary lithium concentration-time curve was reliably separated from the recirculation curve at all levels of exercise, and a pump rate of 30 to 40 mL/min was eventually used. We used this pump speed for measurements during all 7 injections. However, results of a previous study<sup>5</sup> indicated that LiDCO progressively exceeded thermodilution measurements of CO in association with increased duration of use of the lithium sensor. These authors<sup>5</sup> attributed this to either damage to the sensor by excessive flushing, blood clots on the sensor, or binding of lithium to the surface of the sensor. Although results of their study<sup>5</sup> suggested that 8 CO measurements were acceptable for each sensor, that study was performed in anesthetized dogs and therefore a much slower rate of blood was pumped through for each measurement than in the present study. The pump speed used in the present study resulted in 600 to 900 mL of blood flowing through the sensor during each experiment. It is possible that a more optimal combination of lithium dose and pump speed for the lower exercise intensities could be found and that more frequent changing of the sensors would reduce this problem.

Simply limiting the number of CO measurements performed during a short period may allow lithium dilution to be used successfully in the clinical assessment of horses undergoing treadmill examinations for possible cardiac dysfunction. This would reduce peak lithium concentrations, minimize potential increases in lithium background, and limit exposure of the sensor to possible contamination and damage. For clinical evaluation of CO during intense exercise, it is most likely that 1 or 2 measurements at high speed, after a baseline measurement, should be sufficient to evaluate CO in horses with a complaint of poor performance.

Although the lithium dilution method of determining CO is noninvasive, and instrumentation is relatively easy to accomplish, we did find the measurements technically more difficult at high speeds, compared with walking. The computer requires a stable, zeroed baseline before lithium chloride injection; this could not always be accomplished in the short time frames allowed while a horse is galloping at maximal speed on a treadmill. The factors contributing to instability of the baseline at speed could not be precisely determined but could be related to vibration associated with horses running on the treadmill or rapidly changing hematologic conditions during intense exercise. In addition, the lithium dilution method requires a steady-state CO be maintained while the computer generates a primary lithium concentration-time curve. This curve was not always obtained immediately, which could be problematic for a horse maximally exercising. For these reasons, the number of injections that can be performed while a horse is maximally exercising on the treadmill would be limited to 1 or, at most, 2 injections.

In conclusion, lithium dilution may be useful for evaluating CO in maximally exercising client-owned horses. Further studies are needed to determine the optimal combination of lithium dose and pump speed needed for exercise at slower speeds, along with maximum background plasma lithium concentrations, while still obtaining reliable information. The maximum

number of repetitive uses of each sensor also needs to be established, as this may interfere with accurate measurements. Although it cannot be used for multiple measurements in exercising horses and is not a substitute for the direct Fick technique for research purposes, lithium dilution has the potential to provide a reasonable estimate of CO, if the above-mentioned caveats are kept in mind. Further studies evaluating reproducibility and technical feasibility are warranted before it can be considered clinically useful in maximally exercising horses. However, lithium dilution may provide a reasonable alternative in the clinic setting to the use of the invasive and technically demanding method involving the Fick principle.

- a. Classic 4000, Walmanik Inc, Freedom, Pa.
- b. Sigma Chemical Co, St Louis, Mo.
- c. Physitemp Instruments Inc, Clifton, NJ.
- d. DATAQ, Akron, Ohio.
- e. Medical gas analyzer, model 1100, Marquette Gas Analysis Corp, St Louis, Mo.
- f. Model M1420A, Hewlett-Packard Medical Products, Andover, Mass.
- g. Model 200B, Teledyne Hastings-Raydst, Hampton, Va.
- h. OSM-3 radiometer, Copenhagen, Denmark.
- i. YSI 2300, Stat Plus, Yellow Springs, Ohio.
- j. Model 288, Ciba-Corning, Norwood, Mass.
- k. CM 31-01, version 3.4.2, LiDCO Ltd, London, England.
- l. Stata, version 9.2, Stata Corp, College Station, Tex.

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