# Evaluation of accuracy and reliability of indirect calorimetry for the measurement of resting energy expenditure in healthy dogs

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**Objective**—To assess accuracy and reliability of open-flow indirect calorimetry in dogs.

**Animals**—13 clinically normal dogs.

**Procedure**—In phase 1, oxygen consumption per kilogram of body weight ($V_{O_2}$/kg) was determined in 6 anesthetized dogs by use of open-flow indirect calorimetry before and after determination of $V_{O_2}$/kg by use of closed-circuit spirometry. In phase 2, four serial measurements of $V_{O_2}$ and carbon dioxide production ($V_{CO_2}$) were obtained in 7 awake dogs by use of indirect calorimetry on 2 consecutive days. Resting energy expenditure (REE) was calculated.

**Results**—Level of clinical agreement was acceptable between results of indirect calorimetry and spirometry. Mean $V_{O_2}$/kg determined by use of calorimetry before spirometry was significantly greater than that obtained after spirometry. In phase 2, intraclass correlation coefficients (ICC) for REE and $V_{O_2}$ were 0.779 and 0.786, respectively, when data from all 4 series were combined. When the first series was discounted, ICC increased to 0.904 and 0.894 for REE and $V_{O_2}$, respectively. The most reliable and least variable measures of REE and $V_{O_2}$ were obtained when the first 2 series were discounted.

**Conclusions and Clinical Relevance**—Open-flow indirect calorimetry may be used clinically to obtain a measure of $V_{O_2}$ and an estimate of REE in dogs. Serial measurements of REE and $V_{O_2}$ in clinically normal dogs are reliable, but a 10-minute adaption period should be allowed, the first series of observations should be discounted, multiple serial measurements should be obtained, and REE. (*Am J Vet Res* 2001;62:1761–1767).

Malnutrition or overfeeding may adversely affect clinical outcome in critically ill humans,[1] and precise assessment of **resting energy expenditure (REE)** has become an integral part of nutritional support in humans.[2,3] A reliable approach for assessing the daily energy expenditure (kcal/d) is to measure REE under a standard set of conditions.[1,2,4,5] Standard conditions for measurement of basal energy expenditure in humans include measurement in the early morning following an overnight sleep and a fast of at least 10 hours and measurement in a thermoneutral environment devoid of physiologic or psychological stress. In addition, the patient must have a normal body temperature and be in a supine position. Because it is impractical to achieve these conditions for the measurement of basal energy expenditure in hospitalized humans, measurements are made under resting conditions. The resting conditions under which REE is measured are less strictly defined and may include periods of diet-induced thermogenesis, physiologic or psychological stress, and variations in body and environmental temperature.[6] A clear definition of the conditions under which the metabolic rate was measured should always be provided to facilitate comparisons among studies.[4]

Open-flow indirect calorimetry measures gas exchange, specifically **oxygen consumption ($V_{O_2}$)** and **carbon dioxide production ($V_{CO_2}$)**, at the level of the lungs and is the current standard for measuring REE in hospitalized humans.[3,7] These gas exchange measurements are converted to kilocalories per day by application of the abbreviated Weir formula[8,9] or by calculating the caloric equivalent of the liters of oxygen consumed and carbon dioxide produced by use of standardized tables.[6,10] Reliability,[11-13] accuracy,[14-16] and clinical appli cability[1,2,17-19] of indirect calorimetry in humans have been extensively reviewed; however, this technique has just begun to be examined in veterinary medicine.[8,20,21]

The objective of the study reported here was to assess the accuracy and reliability of open-flow indirect calorimetry in healthy dogs. The study was composed of 2 separate but related phases. In the first phase, results obtained by use of the open-flow calorimeter were compared with results obtained by use of the traditional clinical standard, that is, a closed-circuit spirometer for the measurement of $V_{O_2}$. In the second phase, reproducibility of REE and $V_{O_2}$ measurements was assessed in healthy dogs over a 2-day period to determine the reliability of results obtained by use of open-flow indirect calorimetry. We hypothesized that results of the 2 methods used for determination of $V_{O_2}$ would be in agreement and reliability of open-flow indirect calorimetry would be acceptable for clinical use in dogs.

## Materials and Methods

**Animals**—Thirteen adult dogs were used in this 2-phase study. In phase 1, six (3 sexually intact females and 3 sexually intact males) mixed-breed research dogs, ranging in weight

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from 19 to 30 kg and in age from 2 to 3 years, were used to assess the level of agreement between results of open-flow indirect calorimetry and closed-circuit spirometry. Dogs were determined to be clinically normal on the basis of results of physical examination, CBC, and serum biochemical analyses. All dogs were judged to be in good physical condition; none were overweight.

In phase 2, seven (4 spayed females and 3 castrated males) staff-owned dogs of various breeds, ranging in weight from 21.1 to 33.7 kg and in age from 2 to 9 years, were used to determine reliability of REE and $V_{O_2}$ measurements made by use of open-flow indirect calorimetry. No dog had a history of medical problems, and there was no known exposure to anesthesia or exogenous corticosteroids within the previous month. Staff-owned dogs were determined to be clinically normal on the basis of physical examination, CBC, serum biochemical analyses, and free serum thyroxine concentration. In addition, all were within 10% of ideal body weight. These dogs were fed a similar diet[a] and were on a similar feeding schedule prior to and during the study. During the 2-day study period, the time interval between feeding and serial $V_{O_2}$ and $V_{CO_2}$ measurements was consistent for each dog but varied between dogs. For instance, dog 1 ate at 8:00 AM and measurements were performed at 9:00 AM, whereas dog 2 ate at 8:00 AM and measurements were performed at 11:00 AM.

**Equipment**—The commercial open-flow indirect calorimeter[b] we used in both phases of this study measures the partial pressure of oxygen and carbon dioxide in expired air by use of a galvanic fuel cell and a nondispersive infrared analyzer, respectively. Expiratory ventilation volume is measured by use of a flat plate orifice pneumotach. The flow of gas through the sharp-edged thin-plate pneumotach orifice produces a pressure drop proportional to the square root of the flow. This flow signal is then integrated to obtain the expired-air volume. Independent instruments within the system measure temperature, barometric pressure, and time. A fixed proportional sample of the total expired volume, known as a pulse, is drawn into the mixing chamber with each expiration. For each pulse drawn into the mixing chamber, a pulse of identical volume is emitted from the mixing chamber to the oxygen and carbon dioxide sensors. Over a fixed time period, electronic variable sampling allows the pulse trains to be reduced to a constant volume, resulting in similar equilibration times at varying expired flow rates. This system of expired gas sampling is a modification of the breath-by-breath technique. The microprocessor calculates $V_{O_2}$, $V_{CO_2}$, and **respiratory quotient (RQ; $V_{CO_2}/V_{O_2}$)**, using standard equations.[22] In phase 1 of the present study, $V_{O_2}$ and $V_{CO_2}$ were calculated every 20 seconds, and the mean of these readings was printed at 1-minute intervals. The gas analyzers of the open-flow system were calibrated daily, using a standard gas of known composition (18.0% $O_2$, 2.0% $CO_2$, and the balance nitrogen) that was similar to the composition of canine expired air and, prior to each test, room air. In phase 1, a 1-L precision syringe[c] was used to calibrate the pneumotach prior to each test. In phase 2, the gas analyzers of the open-flow system were calibrated daily, using a standard gas of known composition (18.0% $O_2$, 2.0% $CO_2$, and the balance nitrogen). The pneumotach of the open-flow indirect calorimeter was directly attached to an endotracheal tube (phase 1) or a facemask (phase 2) for data collection.

The closed-circuit water-filled spirometer[d] we used in phase 1 was attached to a nondistensible pediatric breathing circuit[e] containing a soda-lime canister to remove exhaled carbon dioxide and a nonrebreathing Y-valve to ensure unidirectional flow. The spirometer was attached to the rebreathing portal of the breathing circuit by use of nondistensible vinyl tubing. A pressure test was performed prior to each spirometry test to ensure air was not leaking within the system. After flushing the system with room air, 100% oxygen was added to the 7-L bellows such that oxygen occupied one-fifth of the bellows volume. Inspired oxygen concentration increased from 21 to approximately 25% to ensure that the dogs did not become hypoxemic when breathing the gas mixture from the spirometer. This was confirmed in a preliminary study, using an airway monitor.[f] Volume displacement of the kymograph paper was calibrated by injecting a known quantity of air through the system, using a volume calibration syringe.[c] The mean value for $V_{O_2}$ was obtained by dividing the change in volume by the change in time noted on the kymograph record. Gas volumes recorded were then corrected to standard conditions (ie, 20 C for temperature, 760 mm Hg for barometric pressure, and as dry gases).

**Phase 1 experimental design**—Food was withheld from dogs for 12 hours, and dogs were premedicated with acepromazine[g] (0.02 mg/kg of body weight, IM) and butrophanol[h] (0.2 mg/kg, IM). Anesthesia was induced with propofol[i] (4 mg/kg, IV) and maintained with a constant rate infusion of propofol (0.2 mg/kg/h or to effect). After induction, dogs were intubated and breathed room air. Rectal temperature was maintained between 37.2 and 38.0 C with a circulating water blanket and fleece covers. At 15-minute intervals, rectal temperature was measured, using a rectal probe,[j] mean arterial blood pressure was determined by use of oscillometry,[k] and oxygen saturation was determined by use of pulse oximetry,[l] using an ear probe applied on the tongue. Once a stable plane of anesthesia was obtained, a 5-minute measurement of $V_{O_2}$ (**pre-indirect calorimeter [pre-IC]** value) was determined by use of the open-flow indirect calorimeter. Oxygen consumption was measured on a per kilogram of body weight basis ($V_{O_2}$/kg) and expressed as ml/min/kg. After a 10-minute rest period, the endotracheal tube was attached to the closed-circuit spirometer and kymograph for 4 minutes for determination of $V_{O_2}$/kg by use of spirometry. After another 10-minute rest period, a second 5-minute $V_{O_2}$/kg measurement (post-IC value) was then obtained with the open-flow indirect calorimeter. The entire procedure was repeated to provide four 5-minute open-flow indirect calorimetry readings (2 pre-IC and 2 post-IC) and two 4-minute closed-circuit spirometry readings interposed between the pre- and post-IC readings. Ambient temperature and barometric pressure were recorded each day.

**Phase 2 experimental design**—Serial $V_{O_2}$ and $V_{CO_2}$ determinations were obtained in a thermoneutral environment (22.0 C)[23] with dogs positioned in lateral recumbency after a period of adaptation to the surroundings. The same 2 individuals (EOT and a student) made all $V_{O_2}$ and $V_{CO_2}$ measurements. Four series of 16-minute readings were obtained from each dog on 2 consecutive days. For each dog, these measurements were performed at consistent times on each day. Each 16-minute reading, which comprised 1 series, was divided into a 10-minute adaptation period to allow dogs to become accustomed to the facemask and collection system and six 1-minute data collection periods. Mean $V_{O_2}$ and $V_{CO_2}$ were calculated as an average of values obtained during the six 1-minute readings. Resting energy expenditure (kcal/d) was then calculated from mean $V_{O_2}$ and $V_{CO_2}$ by use of the abbreviated Weir equation[8,9]:

$$REE = [3.94\ (V_{O_2}) + 1.1\ (V_{CO_2})] \times 1440.$$

Urine nitrogen content and, therefore, protein metabolism are discounted in this equation. In critically ill humans, the use of this equation results in only a 2% error in REE, on average, if protein metabolism is not taken into effect.[24]

**Statistical analyses**—In phase 1, a repeated measure ANOVA, which assumed a constant correlation over time

within an animal, was performed to assess the effect of time on pre- and post-IC values. When a significant effect of time ($P < 0.05$) was detected, a contrast *t*-test was performed. Results were compared between the 2 spirometry readings by use of a paired *t*-test. A limit of agreement analysis, as described by Bland and Altman,[25] was used to determine the level of agreement between pre- and post-IC values and spirometry readings. For comparison with results of other studies, a Pearson correlation coefficient was calculated between pre- and post-IC and spirometer values.

In phase 2, reliability of REE and $Vo_2$ measurements was estimated by calculating **intraclass correlation coefficients (ICC)**. The ICC were estimated from an ANOVA that examined the different variance components (ie, dog, day, and series) and their possible interactions (ie, dog × day, dog × series, and dog × day × series) in a nested-split plot model and accounted for the auto correlation in the data by fitting a time series. Intraclass correlation coefficients are used to evaluate reliability of measurements by estimating the degree of change from 1 measurement to the next for a particular test.[26] An ICC of 1.0 indicates that there is no within-subject variance associated with a test. Conversely, an ICC of zero indicates that there is no reliability associated with a test. An ICC > 0.8 is considered to represent good reliability; however, an ICC > 0.7 is considered acceptable.[5,11,12] Intraclass correlation coefficients were also determined for data obtained in subsets of series (eg, series 2 and 3 or series 3 and 4). Mean values for REE and $Vo_2$ were reported as best linear unbiased estimates. These provide the best estimates of what the actual REE or $Vo_2$ will be for an individual after all variance components (ie, dog, day, series, dog × day, dog × series, dog × day × series) have been taken into account. Best linear unbiased estimates are interpreted in a similar manner to means derived from a fixed-effects model; however, they are the appropriate estimators of means in a random-effects model.[27] Probability values of $\alpha < 0.05$ were considered significant. Statistical analyses were performed, using a commercial software package.[1]

## Results

**Phase 1**—A steady plane of anesthesia was achieved and maintained in all dogs throughout the experiment. Room temperature remained between 21.0 and 22.0 C, rectal temperature between 37.2 and 38.0 C, and mean arterial blood pressure between 60 and 70 mm Hg. Oxygen saturation was > 94% in all dogs.

Time had an effect ($P = 0.08$) on indirect calorimetry measurements. Therefore, a contrast *t*-test was performed to compare pre- and post-IC values. There was a significant ($P = 0.018$) difference between pre- and post-IC values; in both sets of measurements, pre-IC $Vo_2$/kg was greater than post-IC $Vo_2$/kg. The remainder of statistical analyses were thus performed, using the means of the 2 pre-IC and the 2 post-IC readings as separate estimates for $Vo_2$/kg. We did not detect significant ($P = 0.498$; 95% CI, –0.678 to 0.378 ml/min/kg) differences between the 2 spirometry readings; therefore, the mean of these 2 readings was used for statistical analyses.

We detected a significant ($r = 0.950$; $P < 0.001$) correlation between pre-IC $Vo_2$/kg and $Vo_2$/kg determined by use of spirometry. In addition, post-IC $Vo_2$/kg was significantly ($r = 0.863$; $P < 0.001$) correlated with results of spirometry. Bland-Altman plots[25] were derived to compare the difference between results obtained by use of the 2 methods (indirect calorimetry result – spirometry result) and the mean of the 2 results ([indirect calorimetry result + spirometry result]/2; **Fig 1**). The mean difference (bias) ± SD between pre-IC $Vo_2$/kg and $Vo_2$/kg obtained by use of spirometry was 0.283 ± 0.51 ml/min/kg, whereas between post-IC $Vo_2$/kg and $Vo_2$/kg obtained by use of spirometry, bias was 0.13 ± 0.63 ml/min/kg. Inspection of the Bland-Altman plot revealed that no data points fell outside the hatched lines (± 1 ml/min/kg), indicat-

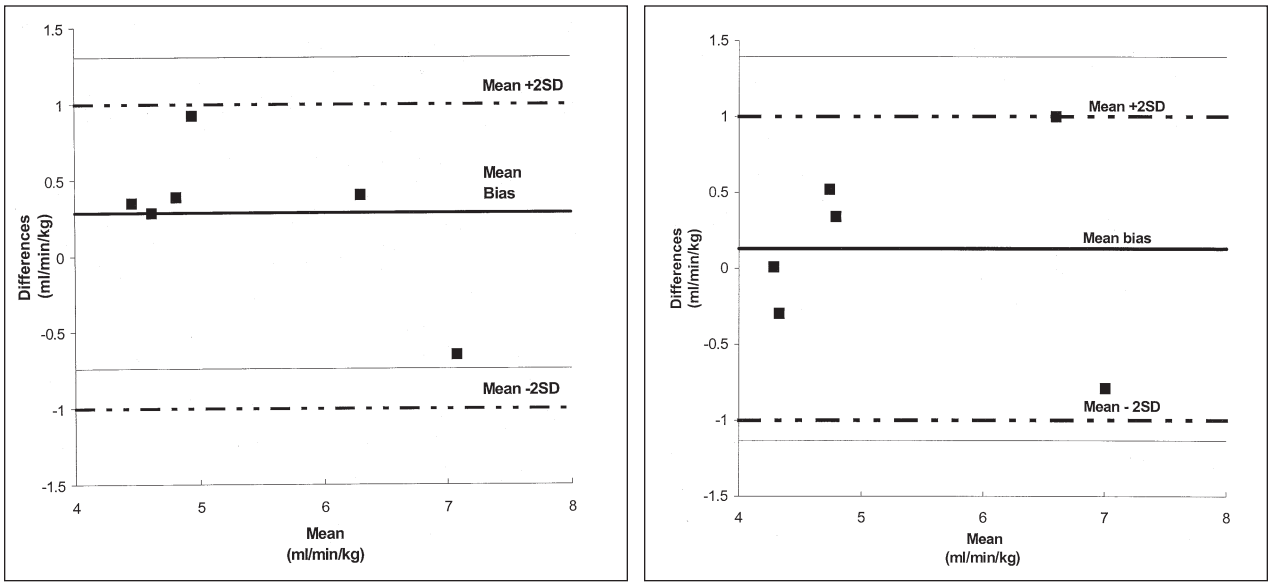


Figure 1—Bland-Altman bias plots comparing the difference in oxygen consumption per kilogram of body weight ($Vo_2$/kg) determined by use of open-flow indirect calorimetry before (left) and after (right) determination of $Vo_2$/kg by use of closed-circuit spirometry versus the mean of values determined by use of both methods. Oxygen consumption was measured in 6 clinically normal anesthetized dogs. The heavy solid line indicates mean bias. The 95% confidence interval around the mean (± 2 SD) is also shown (light solid lines), as well as the predefined target for an acceptable level of clinical agreement (1 ml/min/kg; hatched lines). All data points fall within these hatched lines; therefore, level of clinical agreement between methods is acceptable.

ing that level of clinical agreement between methods was acceptable.

**Phase 2**—Staff-owned dogs appeared relaxed for most of the readings. Minute expiratory ventilation volumes ranged from 2 to 10 L/min. Although no specific attempt was made to record stimuli (eg, increased activity or noise in the surrounding area) or muscular movement (eg, tail wagging, lifting of front paw) that may have resulted in an increase in minute REE, $V_{O_2}$,

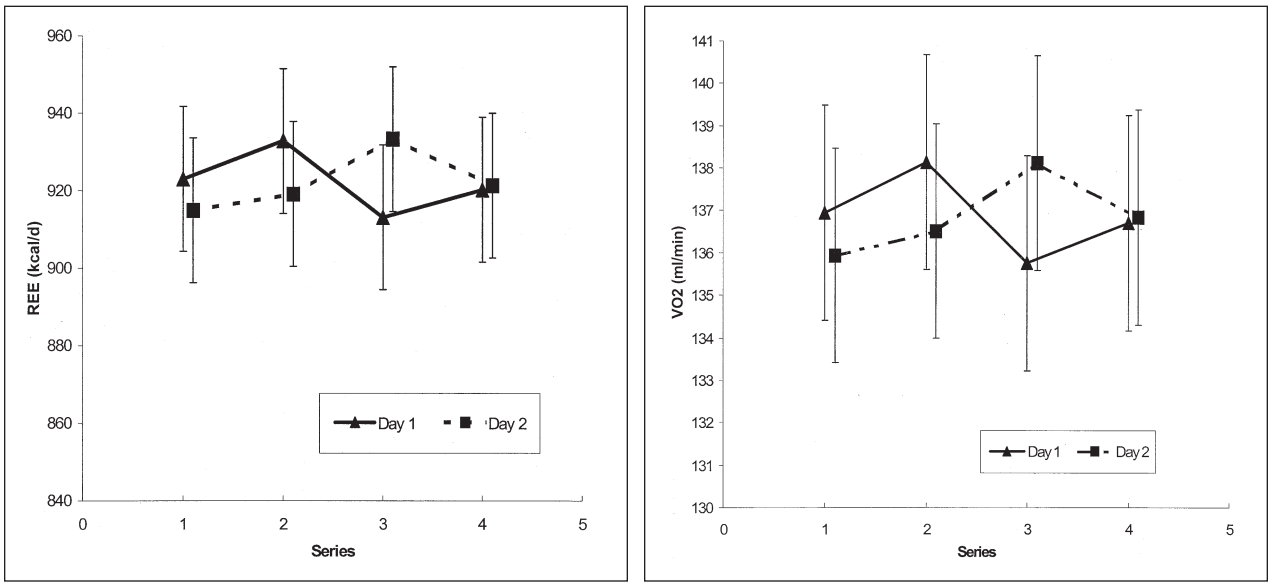


Figure 2—Mean ± SEM resting energy expenditure (REE; left) and oxygen consumption ($V_{O_2}$; right) determined by use of open-flow indirect calorimetry in 7 clinically normal awake dogs on 2 consecutive days. Resting energy expenditure and $V_{O_2}$ were determined over four 16-minute series; a series comprised a 10-minute adaptation period and six 1-minute readings.

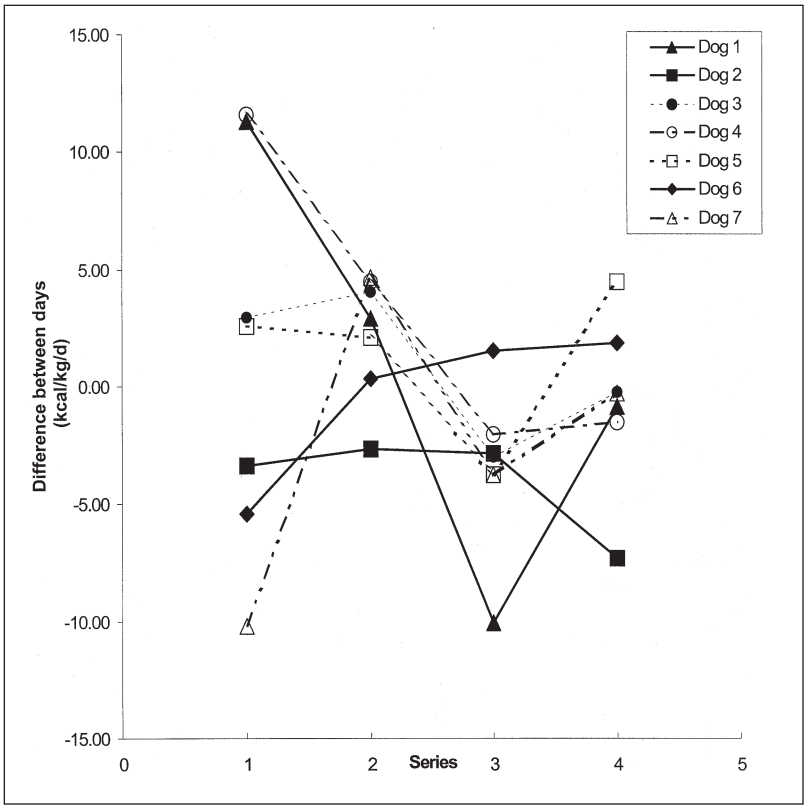


Figure 3—Difference in REE determined on each of 2 consecutive days by use of open-flow indirect calorimetry in 7 clinically normal awake dogs. Resting energy expenditure was determined over four 16-minute series, and each symbol represents the best linear unbiased estimate of REE determined for a given series in an individual dog.

Table 1—Interclass correlation coefficients* (ICC), expressed as a percentage of the total variance and separated according to variance components, for resting energy expenditure (REE) and oxygen consumption ($V_{O_2}$) determined by use of open-flow indirect calorimetry in 7 clinically normal awake dogs on 2 consecutive days

| | Series† | | | |
|---|---|---|---|---|
| **Variance components** | **1–4** | **2–4** | **2–3** | **3–4** |
| REE | | | | |
| Dog‡ | 22.8 | 41.8 | 49.1 | 46.6 |
| Dog × day‡ | 27.0 | 24.7 | 17.0 | 20.7 |
| Series‡ | 35.7 | 21.6 | 22.7 | 21.9 |
| RE‡ | 22.6 | 15.1 | 14.4 | 13.8 |
| Mean of 6 readings§ | 77.9 | 90.4 | 86.7 | 91.1 |
| $V_{O_2}$ | | | | |
| Dog‡ | 23.7 | 43.7 | 50.9 | 48.5 |
| Dog × day‡ | 23.7 | 22.4 | 15.1 | 19.3 |
| Series‡ | 36.4 | 21.1 | 21.5 | 21.2 |
| RE‡ | 25.6 | 16.2 | 15.8 | 14.0 |
| Mean of 6 readings§ | 78.6 | 89.4 | 86.9 | 89.1 |

*Good reliability, ICC ≥ 80%; acceptable reliability, ICC between 70 and 79%; poor reliability, ICC ≤ 69%. †Resting energy expenditure and $V_{O_2}$ determined over four 16-minute series; a series comprised a 10-minute adaptation period and six 1-minute readings. ‡Interclass correlation coefficients determined on the basis of six 1-minute readings. §Interclass correlation coefficients determined on the basis of the mean of six 1-minute readings.

RE= Residual error expressed as a percentage of the total variance

Table 2—Variation (actual residual error) in minute-to-minute and mean REE and $V_{O_2}$ determined by use of open-flow indirect calorimetry in 7 clinically normal awake dogs on 2 consecutive days

| | Series* | | | |
|---|---|---|---|---|
| **Variance components** | **1–4** | **2–4** | **2–3** | **3–4** |
| REE | | | | |
| RE variance min to min† | 12,513.03 | 9,346. | 46 9,291.80 | 9,398.43 |
| Error variance for mean‡ | 9,459.14 | 5,017.00 | 7,328.77 | 5,247.74 |
| $V_{O_2}$ | | | | |
| RE variance min to min† | 309.95 | 217.40 | 218.27 | 205.59 |
| Error variance for mean‡ | 192.65 | 119.38 | 152.31 | 137.82 |

*Resting energy expenditure and $V_{O_2}$ determined over four 16-minute series; a series comprised a 10-minute adaptation period and six 1-minute readings. †Determined on the basis of six 1-minute readings. ‡Determined on the basis of the mean of six 1-minute readings.

RE = Actual residual error variance component as estimated by the proc mixed model.

and ventilation volumes, lower values for these variables in individual dogs generally coincided with sleep or alterations in state of consciousness.

Mean REE and $V_{O_2}$ were calculated for all 4 series on each day (**Fig 2**). Mean RQ for each series on either day was 0.67. The greatest mean absolute difference in REE between days was 20.19 kcal/d in series 3, and the lowest difference was 1.07 kcal/d in series 4. In 1 dog, the difference in REE between days in 1 series was as high as 11.8 kcal/kg/d; however, for most dogs in most series, differences in REE between days were considerably less (**Fig 3**).

Day or series did not have a significant effect on REE ($P$ = 0.99 and 0.82, respectively) or $V_{O_2}$ ($P$ = 0.95 and 0.81, respectively). However, we did detect 2 significant 2-way interactions (dog × day and dog × series) and 1 significant 3-way interaction (dog × day × series). These interactions indicated that REE and $V_{O_2}$ measured in 2 different dogs or in the same dog on different days were not similar. The magnitude of the difference between serial REE and $V_{O_2}$ values varied among series. Moreover, there was unequal variance from day to day over repeated measures in the same dog (Fig 3). However, despite these significant individual day-to-day variations, ICC indicated that overall REE measurements were reliable (**Table 1**). Finally, when all effects were constant (ie, same dog, same day, same series), variation (residual error) in minute-to-minute REE and $V_{O_2}$ was large (**Table 2**).

Reliability of REE and $V_{O_2}$ determinations was considered acceptable (ICC = 0.778 and 0.786, respectively) when data obtained for all 4 series from both days were combined. To obtain a more reliable measure of REE or $VO_2$, data from the first series were discounted, and data from series 2 and 3 or series 3 and 4 were combined. Intraclass correlation coefficients for REE determined after combining series 2 and 3 or series 3 and 4 were 0.867 and 0.844, respectively, whereas ICC for $V_{O_2}$ were 0.915 and 0.908, respectively. Values for REE determined in series 3 (SD, 97.24 kcal/d) and 4 (SD, 97.47 kcal/d) had significantly less variance than values determined in series 1 (SD, 149.19 kcal/d). Therefore, REE determined in series 3 would provide a more accurate estimate of mean REE regardless of day or dog. However, in series 3 and 4, when readings were obtained in a like manner (ie, same day, same dog, same series), 95% of the individual readings could be expected to vary above and below the mean by as much as 195 kcal/d or, on average, by 7.27 kcal/kg/d. Dividing the SD by the mean body weight of the dogs enrolled in the study translated the units to kcal/kg/d.

## Discussion

Results of the first phase of our study revealed a high degree of correlation ($r$ = 0.86 to 0.95) between open-flow indirect calorimetry and closed-circuit spirometry. More importantly, Bland-Altman analysis revealed that results of these 2 methods were in good agreement.[25] However, because correlation is a measure of association and not agreement, it cannot be used by itself to determine how interchangeable the 2 methods are for assessing an individual dog. Instead, determination of the limits of agreement is a more appropriate test of agreement between 2 methods of a clinical measurement.[25] We chose a level of agreement of 1 ml/min/kg between the 2 methods we used to determine $V_{O_2}$/kg. This level of clinical agreement was based on results of a pilot study and results of previous studies in dogs[8,21,28,29] and represented approximately 15% of REE in healthy dogs.

Because we found excellent agreement between results of open-flow indirect calorimetry and closed-circuit spirometry, we believe that these methods can be used interchangeably to measure $V_{O_2}$ in dogs. Historically, closed-circuit spirometry has been considered the standard method for measurement of $V_{O_2}$. The advantage of this technique is that no oxygen or flow-measuring devices are required, nor is there a need to use the Haldane transformation.[30] However, any leaks in the system will result in inaccurate measurements of $V_{O_2}$. Furthermore, to prevent hypoxemia, a high concentration of oxygen is added to the inhaled gas mix-

ture. This may limit the data collection time depending on the size of the spirometric bellows, because there may be insufficient time for the patient's carbon dioxide and oxygen stores to reach equilibrium.[30] Also, the addition of a carbon dioxide absorber into the circuit may result in an increase in airway resistance and expired air temperature over prolonged test periods. For all potential purposes, closed-circuit spirometry will only provide an accurate measure of $V_{O_2}$ in intubated dogs.

Open-flow indirect calorimetry provides a more flexible measurement system for determination of REE in a clinical setting, because most hospitalized dogs do not require intubation or high inspired oxygen concentrations. The open-flow system we evaluated cannot be used in dogs receiving supplemental oxygen. However, in most clinical situations, supplemental oxygen could be discontinued during the test.[22] In the present study, pre- and post-IC $V_{O_2}$/kg values were significantly different. This difference may have been attributable to the establishment of a more steady metabolic state during the second open-flow indirect calorimetry measurement (ie, the post-IC reading). However, we are not sure why pre- and post-IC values differed. If there had been any residual increase in alveolar concentration of oxygen after obtaining the spirometry reading, the effect would have been to increase the post-IC $VO_2$/kg value. Instead, a decreased value was observed in this study.

Reliability analysis indicated that despite the inherent variability of REE measurements, the reliability of the serial open-flow indirect calorimetry for determination of REE was acceptable. There was significant variation in results for individual dogs from series to series and from day to day. However, when results were averaged over all dogs, day or series did not have a significant main effect on REE, indicating that measurements were repeatable between days in this group of clinically normal dogs. The pattern of variance in data obtained from the first series of measurement differed from that in data obtained from the other series. In addition, variance in the first series was higher. These differences have been observed by others studying dogs[8] or humans[11,12] and may be related to behavioral changes of the test subject. Behavioral changes in the dogs of this study may have been the result of acclimation to the facemask and collection system. Therefore, discounting data from the first 2 series and analyzing data from the last 2 series resulted in more reliable estimates of REE in the present study. This observation will probably be valid for subsequent clinical use of indirect calorimetry for determination of REE in dogs.

Results of a previous study[8] also revealed good reliability of serial indirect calorimetry readings. In that study, 5 evaluations were performed in 20 clinically normal dogs over an 8-hour period. Reliability of energy expenditure per kilogram of body weight data was better when the middle 3 readings were averaged (ICC, 0.87) than when all 5 evaluations were averaged (ICC, 0.02) or when the first evaluation was eliminated (ICC, 0.46). The authors concluded that to obtain a reliable estimate of energy expenditure, a 15-minute adaptation period should be allowed so dogs can become accustomed to the collection system, the first reading should be discounted, and subsequent readings should be averaged for data analysis.

We anticipated that the manner in which we collected data in phase 2 would enable us to measure REE and $V_{O_2}$ in a steady state. However, despite our dogs appearing rested and relaxed during data collection, we still detected considerable variability in data obtained from individual dogs from series to series or from day to day. In human medicine, the definition of a patient's resting state is characterized by a consistent coefficient of variation over a predefined time (ie, a steady state) and is not solely based on clinical observation.[31] We chose not to eliminate any readings in the present study on the basis of a predefined coefficient of variation. However, etablishing such exclusion criteria would have improved repeatability of REE and $V_{O_2}$ determinations. A steady metabolic state implies that gas exchange measurements are equal to tissue gas exchange. It is under these conditions that the assumptions associated with indirect calorimetry are valid.[18,32] Therefore, some of the variation and the lower RQ that we detected may have been attributable in part to a failure of our dogs to achieve a steady metabolic state. Also, the open-flow indirect calorimeter has been reported to underestimate $V_{CO_2}$ at lower expired ventilation volumes in humans.[33] Therefore, at low ventilation volumes (2 to 10 L/min), as were detected in the present study, an underestimation of $V_{CO_2}$ would have been likely, resulting in low RQ. This underestimation may have been attributable to the proportional sampling technique that is inherent to the indirect calorimeter.[22] However, even if large errors in the distribution of energy production among the 3 fuels (ie, fat, carbohydrate, and protein) were present, there would have been little effect on total energy expenditure, because the caloric equivalent of oxygen varies by $< 15\%$.[24]

The range of $V_{O_2}$ measured in the present study (4.15 to 6.40 ml/min/kg) was comparable to values reported by others in resting dogs (6.0 to 12.3 ml/min/kg).[8,28,29,34] The range of REE (17.28 to 63.62 kcal/kg/d) was also comparable to values reported by Walters et al[8] (38.72 to 50.42 kcal/kg/d) and Galvao[28] (31.44 to 57.84 kcal/kg/d). Variation in the level of consciousness of the dogs during data collection periods may have accounted for the wide range of values in our study. Peters et al[29] reported a similar fluctuation in $V_{O_2}$ measured by use of indirect calorimetry in dogs. In that study, a substantial difference in mean $V_{O_2}$ was detected, which was deemed attributable to the dog's level of awareness. In a resting state, $V_{O_2}$ fluctuated between 2 and 10 ml/min/kg (mean ± SD, 5.57 ± 1.18 ml/min/kg). Mean $V_{O_2}$ decreased to 3.97 ± 1.01 ml/min/kg when dogs were drowsy and was at its lowest and least variable when dogs were asleep (2.46 ± 0.48 ml/min/kg). Therefore, state of consciousness should be recorded during measurement of $V_{O_2}$ by use of open-flow indirect calorimetry in dogs, and attempts should be made to ensure that all dogs remain in a similar state of awareness throughout data collection.

We found that in healthy adult dogs, $V_{O_2}$/kg measured by use of open-flow indirect calorimetry was in

agreement with values determined by use of spirometry. Therefore, the open-flow indirect calorimeter may be used in most clinical settings to obtain a measure of $V_{O_2}$ and, moreover, an estimate of REE in dogs. Serial measurements of REE and $V_{O_2}$ in clinically normal dogs obtained over 2 consecutive days were found to be reliable. This was particularly true if a 10-minute adaption period was allowed, the first series of observations was discounted, multiple serial measurements were obtained, and only the last 2 serial measurements were used to calculate REE.

[a]Eukanuba maintenance, Iams Co, Dayton, Ohio.
[b]TEEM 100 metabolic apparatus, Aerosport Inc, Ann Arbor, Mich.
[c]1-L calibration syringe Model 5540, Hans Rudloph Inc, Kansas City, Mo.
[d]Benedict-Roth spirometer, Warren E. Collins Inc, Braintree, Mass.
[e]Bloomquist infant circle absorber system, Forgger Co, Roslyn Heights, NY.
[f]Critcare, Poet IQ Inc, Waukesha, Wis.
[g]Atravet, Ayrest Laboratories, Montreal, QC, Canada.
[h]Torbugesic, Ayrest Laboratories, Montreal, QC, Canada.
[i]Rapinovet, Schering-Plough, Pointe-Claire, QC, Canada.
[j]Sona Temp 400/700 monitor, Sheridan Catheter Corp, Arygle, NY.
[k]Dinamap, Critikon Inc, Tampa, Fla.
[l]General linear and Proc mixed models, SAS Institute Inc, Cary, NC.

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