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Direct calorimetry reveals large errors in respirometric estimates of energy expenditure

Glenn E. Walsberg* and Ty C.M. Hoffman
School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA
*Author for correspondence (e-mail: walsberg@asu.edu)

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Summary

Knowledge of animal energetics is based largely upon indirect calorimetry, which is estimation of metabolic heat production by an organism from measurement of indices such as oxygen consumption or carbon dioxide production. Remarkably, indirect calorimetry has been validated by comparison to direct measurements of metabolic heat production (direct calorimetry) only for highly restricted conditions, primarily with a few species of medium-to-large mammals. Taxa with differing physiologies are little studied. For birds, for example, validations are limited to chickens and waterfowl exposed to mild environmental conditions and typically fasted for prolonged periods. Workers rely upon these restricted validations when studying animals ranging greatly in activity, phylogeny, body size and nutritional status. We tested the accuracy of respirometric estimates of energy expenditure by simultaneous indirect and direct calorimetry in a small mammal (the kangaroo rat *Dipodomys merriami* Mearns), a small bird (the dove *Columbina inca* Lesson) and a medium-sized bird (the quail *Coturnix communis* Linnaeus). We find that conventional respirometric estimates of energy expenditure may incorporate large errors (up to 38%) that are sufficient to call into question generalizations regarding patterns of animal energy use in many studies.

Key words: calorimetry, respirometry, metabolic power consumption, energy expenditure, kangaroo rat, *Dipodomys merriami*, Inca dove, *Columbina inca*, quail, *Coturnix communis*.

Introduction

Because energy relations have such broad significance for organismal function, use of chemical potential energy and its consequent metabolic heat production is a major theme in modern biology. Animal energy usage is most commonly quantified by indirect calorimetry, which is the estimation of metabolic heat production from measurement of substances consumed (e.g. O_2) or produced (e.g. CO_2) by metabolism. This set of techniques is broadly used in medicine, medical research, agricultural research, exercise physiology and environmental physiology. Indirect estimates of animal metabolic heat production rely, however, upon an extensive chain of assumptions linking them to Hess's Law of Constant Heat Sums. Hess's Law notes that the heat released in a chemical reaction depends only on the nature of the initial reactants and final products, and not on intermediate steps. Therefore, aerobic heat production in animals can be determined from knowledge of the substrate catabolized, combined with measurement of oxygen consumption or carbon dioxide production. In practice, the rate ($l\,s^{-1}$) of oxygen consumption or carbon dioxide production is measured, then multiplied by the estimated thermal equivalent (h; $J\,l^{-1}$) of this respiratory gas exchange.

However, the thermal equivalents of both oxygen consumption (h_{O_2}) and carbon dioxide production (h_{CO_2}) are not constants and must be estimated. Their values depend, for example, upon the substrate's proportional representation of lipids, carbohydrates, and proteins (Table 1). A major issue, therefore, is the task of identifying the proportions of these materials being catabolized. This is typically accomplished using one of three assumptions. These are that the animal is either (a) catabolizing only lipids when fasting (e.g. Walsberg, 1977; Weathers and Sullivan, 1993), or (b) catabolizing materials in proportion to their abundance in the diet (e.g. Benabib and Congdon, 1992; Lindstrom et al., 1993; Konarzewski and Diamond, 1994), or (c) catabolizing materials with a carbohydrate: lipid ratio indicated by the respiratory exchange ratio, RER (the ratio of CO_2 released by the animal to O_2 consumed by it) (e.g. Duncan and Lighton, 1994; Hansen et al., 1995; Lighton and Fielden, 1995). The third of these three assumptions is based upon the contrasting RER values characteristic of each nutrient class. Assuming that animals conserve body stores of protein when fasting, the proportional reliance upon carbohydrates and lipids is calculated using the experimentally observed RER and a linear interpolation between the maximum expected value of RER=1.00 for carbohydrates and the minimum expected value of RER=0.71 for typical dietary lipids. This allows calculation of the net thermal equivalent of the substrate.

Table 1. *Typical RER values and conventionally assumed thermal equivalents of gas exchanges for major classes of metabolic substrates*

	Lipid*	Carbohydrate*	Protein (mammals)*	Protein (birds)†
RER	0.71	1.00	0.81	0.74
h_{CO_2} (kJ l^{-1})	27.8	21.1	23.8	25.4
h_{O_2} (kJ l^{-1})	19.8	21.1	19.2	18.7

Values taken from *Brouwer (1957); †King (1957).

Problematically, these assumptions used to estimate h_{CO_2} and h_{O_2} can readily be violated. First, the catabolic substrate may differ from that expected. Fasting animals may rely on non-lipid substrates, for example, by catabolizing body protein or food stored within the gut (e.g. Ankney et al., 1991). The time required to become postabsorptive is unknown for many species and varies between taxa and with changes in environmental conditions and diet (Robbins, 1993). The assumption that catabolic substrates contain the same nutrient composition as dietary intake subsumes the implicit premise that such well-known processes as differential digestion, absorption, and utilization of nutrients are negligible (Gessaman and Nagy, 1988; Karasov, 1990). A second basis for violating the common assumptions used to estimate h_{CO_2} and h_{O_2} is that the animal may simultaneously be involved in synthetic processes that consume or produce either CO_2 or O_2. Finally, incomplete substrate oxidation can occur (Kleiber, 1961; McLean and Tobin, 1987). This could, for example, include production of ketone bodies during lipid catabolism.

Given the central importance of energy metabolism and the complexity of the physiological assumptions incorporated, it is striking that modern calorimetric methods have not been adequately validated. Estimates derived from indirect calorimetry have been compared to those derived by actually measuring metabolic heat production (direct calorimetry) only for conditions that were highly restricted in ways that importantly affect energy metabolism. Most of these experiments were conducted between 1900 and 1940, and primarily focused on a few species of medium-to-large mammals (McLean and Tobin, 1987). Other taxa were little studied, despite differences in their physiology. Validations are lacking for some groups of major interest (e.g. insects), and validations for other important groups are severely limited. For birds, for example, experiments confirming the adequacy of indirect calorimetry are few, and dealt only with large forms (domestic ducks, geese, and chickens), representing just two taxonomic orders (Hari, 1917; Hari and Kriwuscha, 1918; Benedict and Lee, 1937; Barott et al., 1938; Barott and Pringle, 1941, 1946). These studies were further restricted in that animals were typically fasted for periods from 12 h to 42 days prior to measurements and exposed to mild environmental conditions (environmental temperatures typically were 16–32°C). Finally, measurements were averaged over long periods (4–24 h).

In sharp contrast, modern workers rely upon these limited validations when studying animals of widely varying phylogeny, body mass and nutritional status. Animals may be exposed to extreme temperatures and may range in activity from torpor to vigorous exercise. Finally, metabolic measurements are often made over short periods, and some include calculations of instantaneous metabolic rates.

Our concerns regarding the inadequacy of validations of indirect calorimetry in the face of the technique's extensive use were amplified by the occurrence of respiratory gas exchange patterns that deviated substantially from conventional assumptions regarding energy metabolism. Several laboratories have now demonstrated prolonged maintenance of RER values significantly below the theoretically expected minimum of 0.71 in taxa as diverse as birds and insects (Walsberg and Wolf, 1995; Hawkins et al., 2000; Chaui-Berlinck and Bicudo, 1995; Harrison et al., 1991). Given the broad reliance upon respirometric estimates of metabolic power consumption, we therefore simultaneously measured respiratory gas exchange and metabolic heat production by direct calorimetry in a small mammal (the kangaroo rat *Dipodomys merriami;* mean mass=37.6 g) a small bird (the dove *Columbina inca;* mean mass=38.8 g), and a medium-sized bird (the quail *Coturnix communis;* mean mass=158 g). This allowed us to directly determine the thermal equivalents of oxygen consumption and carbon dioxide production, and to compare these with values commonly used by other workers.

Materials and methods

Animal use

Quail *Coturnix communis* L. were purchased from local aviculturalists. Doves *Columbina inca* Lesson and kangaroo rats *Dipodomys merriami* Mearns were collected from the wild under permits from federal and state authorities, and all research was conducted under the auspices of the Arizona State University Institutional Animal Care and Use Committee. Trials were conducted on 10 individuals of each of the three species. For 24 h prior to the start of each trial, the animal was provided with water and rice *ad libitum*. Each trial lasted at least 17 h, during which the animal was kept in darkness in the calorimetry chamber, at 25°C ambient temperature (T_a), with no access to food or water. To eliminate the possibility that interspecific differences in direct calorimetric measurements were due to drift in the response of the calorimeter, we interspersed trials of each species between trials of the other two species. All trials began between 13:00 h and 15:00 h.

Direct calorimetry

We constructed a combined direct calorimeter/respirometry chamber that allowed simultaneous measurements of animal heat production and respiratory gas exchange. We calculated animal heat production as the sum of conductive heat flux through the surface of the calorimetry chamber, convective heat flux (from the product of air temperature change, the specific heat capacity of the air, and air flow rate), and evaporative

heat flux (from the product of air flow rate, hygrometric measurements, and latent heat of vaporization). Note that these modes of heat transfer refer to heat loss from the metabolic chamber and not from the animal. For example, heat conducted through the chamber walls may have been initially transferred from the animal by conduction, convection or radiation.

We measured conductive flux through the six faces of the calorimetry chamber. The chamber (20 cm×20 cm×20 cm) was constructed of 3 mm thick aluminium. A copper-constantan thermopile was affixed to the exterior of each face, and the six thermopiles were wired in series to create a single thermopile to sum the heat flux across the entire surface of the chamber. Thermopiles were created by winding constantan wire (0.3 mm diameter) through perforated PC board and electroplating copper onto half of each turn of wire. This resulted in each turn of wire having a superficial half and a deep half, each of which contained a copper-constantan (type T) thermocouple junction. The overall thermopile contained approximately 3000 pairs of thermocouples, each pair separated by the thickness of the PC board (1.6 mm). Thermocouple pairs were evenly distributed over each face of the calorimeter. Each face's thermopile was encased in polyester resin (ca. 4 mm thickness) to cover all wires. We affixed the thermopile to the exterior, rather than interior, surface to reduce errors associated with any small areas of localized heat flux that might occur. Heat flowing outwardly (the situation during trials) first flowed through the aluminium walls, which were much more thermally conductive than the outer shell of polyester resin and PC board. Thus, heat was transversely distributed within the plane of the wall before reaching the thermopile, reducing the probability that heat flux occurring between the closely spaced thermocouples was different from that occurring at the thermocouples.

Accurate measurement of conductive flow required that the outer chamber surface be maintained at a constant temperature. During animal trials and thermopile calibrations, the chamber was submerged in a 200 l water bath that was continuously stirred and maintained by proportional thermostatic control at 25.000±0.005°C. To provide feedback for the temperature-control system, we submerged six copper-constantan thermocouples throughout the water bath, each referenced to an ice slurry.

We calibrated the calorimeter's thermopile by placing into the chamber a resistive heater whose heat flux was calculated from measurements of electrical current through, and electrical potential difference across, the resistor. Measurements were made using 10 levels of heat input distributed over the range of 0–1.7 W, which includes the experimentally observed range of rates of animal heat production. We conducted the calibration discontinuously, over several days, both to ensure equilibration at each level of heat flux and to demonstrate repeatability with respect to linearity. Thermopile response was highly linear (r^2=0.998) and allowed resolution of heat production to 0.8 mW.

Convective heat loss was calibrated separately from conductive loss because small changes in the temperature of air entering the chamber could alter the proportions of the total heat that was lost by conduction and convection. Measurement of convective heat loss from the calorimeter was based on the change in the temperature of air flowing through the chamber and the rate of air flow. Air flow rate was maintained at 1234 ml min^{-1} by a mass-flow controller (Unit Instruments UFC-2550, Yorba Linda, CA, USA) that we calibrated using soap-film flowmeters. An inlet and an outlet port allowed flow-through of air that was first dried and scrubbed of carbon dioxide by an industrial air purifier (PureGas model CDA 1112, Westminster, CO, USA). Each port was fitted with a precision thermistor (Omega Engineering 44000 Series, Stanford, CT, USA) to measure air temperature. To measure the response of the thermistors, we constructed bridge circuits using ultra-high-precision resistors (5.774 kΩ ±0.01%). The bridges were controlled by a datalogger (Campbell Scientific 21x, Logan, UT, USA) with a resolution of 0.3 μV. We calibrated the thermistors at several temperatures, in water, to yield polynomials employed in the datalogger during trials.

We quantified evaporative heat loss from the chamber using the known flow rate of air and the increase in water vapor content of effluent air sent through non-hygroscopic tubing (Bev-A-Line V, Thermoplastic Processes Inc., Stirling, NJ, USA) to a dewpoint hygrometer (Sable Systems RH200, Las Vegas, NV, USA). We calibrated the hygrometer using bottled nitrogen (for the zero gas) and air bubbled though ca. 450 vertical cm of temperature-controlled water (for the span gas).

Indirect calorimetry

We used flow-through respirometry to measure oxygen consumption and carbon dioxide production, concurrent with direct measurements of heat production. For maximum accuracy of gas measurements, enough dry, CO_2-free air for an entire trial was pressurized and stored in an air receiver (760 l) prior to each trial. This ensured that air entering the calorimetry/respirometry chamber did not change in composition over the course of the long trials. Additionally, separate baseline values for gas composition were recorded before and after each trial. Air flow, as described above, was maintained at 1234 ml min^{-1}. Air exiting the dewpoint hygrometer was dried by anhydrous calcium sulfate before flowing through a carbon-dioxide analyzer (Li·Cor LI-6252) and then an oxygen analyzer (Sable Systems FC-1B), both of which were calibrated, using bottled gases, at high and low extremes that exceeded those of measurements. The overall system was then verified against a known combustion reaction. A measured mass of 100% ethanol was burned for 6 h inside the calorimetry chamber. Measurements of water vapor, CO_2 production and O_2 consumption were within 0.4% of values expected from the stoichiometry of ethanol combustion. For animal trials, we minimized already miniscule amounts of analyzer drift by calculating oxygen consumption each minute based on oxygen baseline values that, each minute, were linearly interpolated between beginning and ending baseline values.

Gas exchange, heat flux and thermal equivalents

For the three gases that were analyzed, we calculated the volumetric rates of change using the following:

$$\dot{V}_{O_2} = \frac{R_B[F_O(1-F'_C-F'_W) + F'_O(F_C+F_W-1)]}{F_C + F_O + F_W - 1}\,, \quad (1)$$

$$\dot{V}_{CO_2} = \frac{R_B[F_C(F'_O+F'_W-1) + F'_C(1-F_O-F_W)]}{F_C + F_O + F_W - 1} \quad (2)$$

and

$$\dot{V}_{H_2O} = \frac{F'_W R_B - F_W(R_B+\dot{V}_{CO_2}-\dot{V}_{O_2})}{F_W - 1}\,, \quad (3)$$

where $\dot{V}$ denotes rate of flow ($l\,s^{-1}$) of gases indicated by subscripts; R_B is the rate of flow or air into the chamber; F_O, F_C and F_W are the effluent air's fractional content of O_2, CO_2 and H_2O, respectively; and corresponding symbols with primes denote respective fractional contents in the influent air. We calculated conductive heat flux directly from our empirical calibration equation, which is a linear fit of heat flux (mW) through the thermopile as a function of electrical potential difference (mV) measured across the thermopile. For the other two heat fluxes, convective and evaporative, we used the following:

$$\begin{aligned} Q_{conv} = {} & \dot{V}_{H_2O}\rho_s c_{p,s} T_{a,out} \\ & + (\dot{V}_{in,dry}+\dot{V}_{CO_2}-\dot{V}_{O_2})\rho_{dry} c_{p,dry} T_{a,out} \\ & - \dot{V}_{in,dry}\rho_{dry} c_{p,dry} T_{a,in} \end{aligned} \quad (4)$$

and

$$Q_{evap} = \lambda[(\dot{V}_{in}+\dot{V}_{H_2O}+\dot{V}_{CO_2}-\dot{V}_{O_2})\rho_{v,out} - \dot{V}_{in}\rho_{v,in}]\,, \quad (5)$$

where ρ_s and ρ_{dry} are the densities ($kg\,l^{-1}$) of water vapor and dry air, respectively; $c_{p,s}$ and $c_{p,dry}$ are the isobaric specific heat capacities ($J\,kg^{-1}\,deg.^{-1}$) of water vapor and dry air, respectively; $T_{a,out}$ and $T_{a,in}$ are the temperatures of effluent air and influent air, respectively; λ is the latent heat ($J\,kg^{-1}$) of vaporization of water; and $\rho_{v,out}$ and $\rho_{v,in}$ are the vapor densities ($kg\,l^{-1}$) of effluent and influent air, respectively.

We assessed the temporal response of the thermopile by plotting signal output *vs* time after a change in heat flux from the calibration resistor. The output was allowed to reach its equilibration value and remain there for hours. We then calculated the time required for the thermopile's output to reach 99% of its equilibration value, which occurred at 28 min after a sudden change in heat flux. We performed a similar calculation for the time required for the fractional content of CO_2 to reach 99% of its equilibration value following a sudden change in the composition of influent air. The airflow rate used in the experiments was chosen so that the two periods of 99% equilibration (thermopile and gas fraction) were equal. We calculated thermal equivalents as the ratio of total heat flux to $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$, averaged over 30 min. Because 30 min exceeds the periods of 99% equilibration, the mean values are independent.

Statistical analyses and error estimates

We determined empirical values to be statistically different from conventionally expected values if the empirical 95% confidence limits did not overlap the expected range. We tested for progressive changes in thermal equivalents of respiratory gas exchange with time during experimental trials by applying linear regression. The significance of the slope of the regression equation was tested by analysis of variance (ANOVA) and accepted as significant if $P<0.05$.

We calculated mean absolute errors with respect to two common approaches to estimating thermal equivalents. One of these is to assume that body protein is conserved and thus to use RER to linearly interpolate between equivalents for combustion of typical dietary lipid and carbohydrate (Table 1). These calculations probably underestimate actual errors produced by linear interpolation, as h_{CO_2} and h_{O_2} are impossible to calculate when RER values are less than 0.71 or greater than 1.00 (Table 1). The second approach for which mean absolute errors were calculated is the common assumption that fasting animals rely entirely upon lipid catabolism and thus exhibit thermal equivalents equal to those of a mixed lipid substrate (Table 1). In both cases, the errors arising from such estimates were calculated as the absolute value of $100\times[1-(h_{EST}-h_{OBS})/h_{OBS}]$, where h_{EST} is the estimated value and h_{OBS} is the empirically observed value.

Results

Kangaroo rats increased carbon dioxide production, oxygen consumption, and metabolic heat production during the night (approximately 7–15 h after chamber entry between 14:00 h

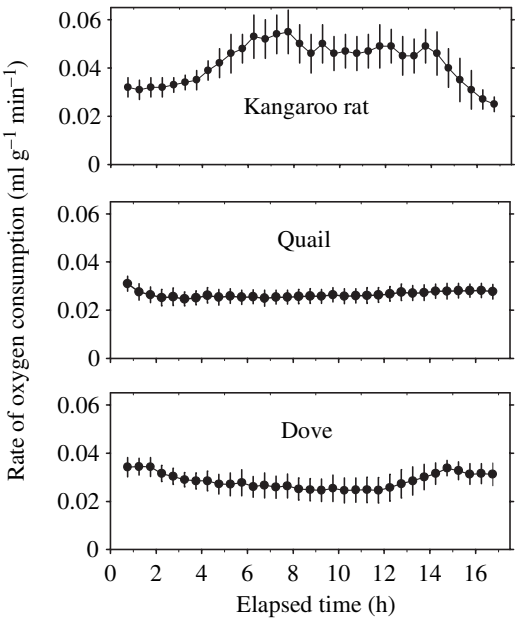

Fig. 1. Rate of oxygen consumption during 17 h after removal from food. Values are means ± 95% confidence intervals; N=10 for each species.

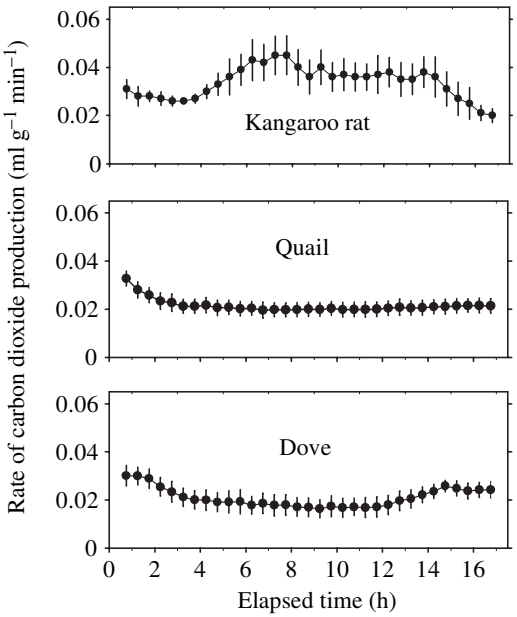

Fig. 2. Rate of carbon dioxide production during 17 h after removal from food. Values are means ± 95% confidence intervals; *N*=10 for each species.

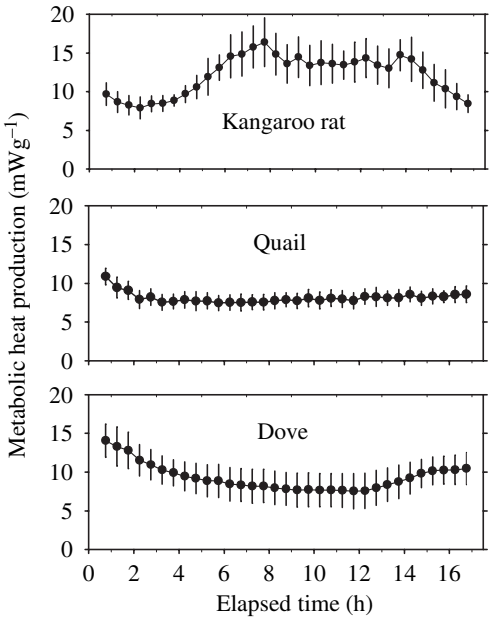

Fig. 3. Metabolic heat production measured by direct calorimetry during 17 h after removal from food. Values are means ± 95% confidence intervals; *N*=10 for each species.

and 15:00 h), whereas these rates in quail and doves either remained relatively constant or declined (Figs 1–3). This difference presumably reflects the difference in these species' activity periods; kangaroo rats are nocturnal and both bird species are diurnal.

Respiratory exchange ratios (RER) in all species were initially near or above 1.00, suggesting extensive reliance upon carbohydrate as a catabolic substrate (Fig. 4). Values of

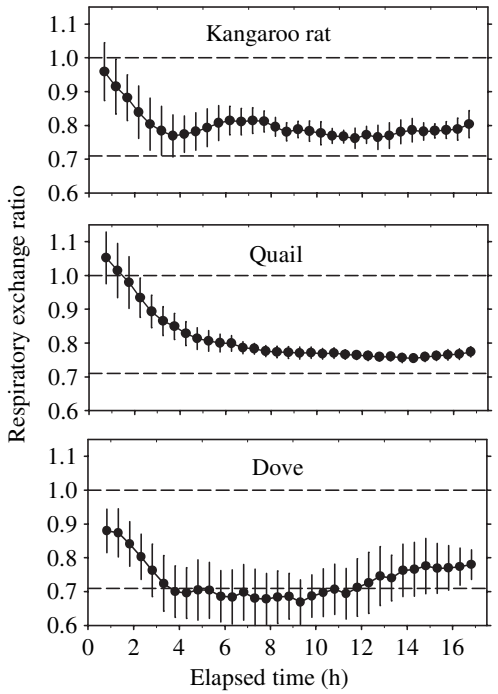

Fig. 4. Respiratory exchange ratios during 17 h after removal from food. Values are means ± 95% confidence intervals; *N*=10 for each species. Broken lines indicate expected maximum and minimum values.

1.01–1.20 were observed in individuals of all species during the first 4 h of experimental trials; this was exhibited by four kangaroo rats, five quail and one dove. Over the course of 4–5 h of fasting, RER declined and averaged 0.78 in kangaroo rats and 0.76 in quail during the last 10 h of measurements. In doves, RER also declined and averaged 0.76 during the middle of experimental trials (hours 7.5–9.0) but increased during the latter portion of trials. During the final 7 h of experimental trials, three kangaroo rats exhibited RER values of 0.65–0.70, below the minimum anticipated for exclusive reliance upon lipids as a metabolic substrate in a fasting animal. Unexpectedly low (0.58–0.69) RER values were also seen in four doves during hours 3–14 of trials.

Measured thermal equivalents of consumed oxygen (h_{O_2}) were commonly outside the conventionally expected range (Fig. 5). Mean h_{O_2} in kangaroo rats was significantly below its commonly assumed minimum during 21 of 33 intervals, and tended to increase with time ($P<0.05$). Doves exhibited nearly the opposite pattern; mean h_{O_2} initially exceeded expected maximum values and tended to decrease with time ($P<0.05$), although the three final 30–min periods returned to values significantly above the expected maximum. Quail exhibited an intermediate pattern. After the initial 3 h of the experimental trial, mean h_{O_2} exhibited no significant change with time ($P=0.97$). Although h_{O_2} was below its conventionally assumed minimum during 27 of the 33 half-hour intervals, the expected minimum was within the 95% confidence limit of empirical data during all but two intervals.

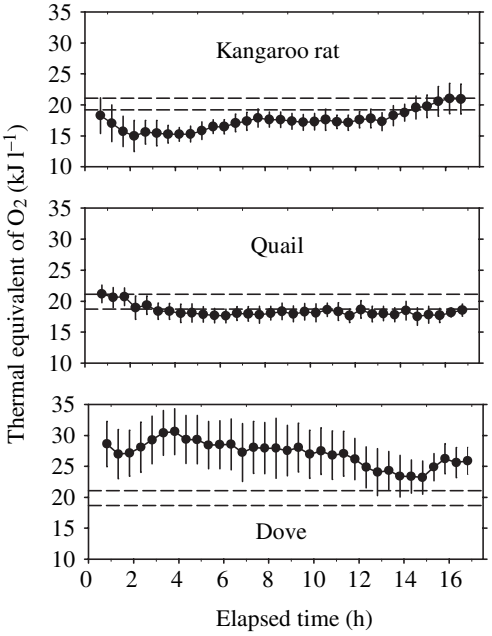

Fig. 5. Thermal equivalents of oxygen consumption during 17 h after removal from food. Values are means ± 95% confidence intervals; *N*=10 for each species. Broken lines represent the expected maximum and minimum values (Table 1).

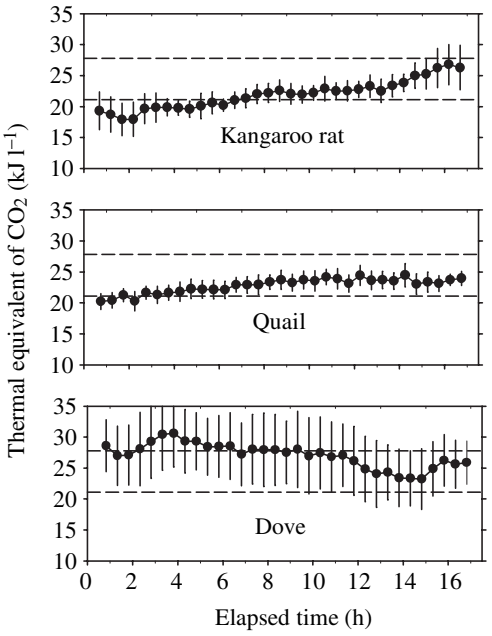

Fig. 6. Thermal equivalents of carbon dioxide production during 17 h after removal from food. Values are means ± 95% confidence intervals; *N*=10 for each species. Broken lines represent the expected maximum and minimum values (Table 1).

The mean thermal equivalent of produced carbon dioxide tended to increase with time in kangaroo rats ($P<0.05$) and was below its conventionally assumed minimum during 12 of the 33 half-hour intervals (Fig. 6). During four of 33 intervals, the expected minimum was above the 95% confidence limit of empirical data. Mean h_{CO_2} in doves tended to decrease with time ($P<0.05$) and included values that were below the expected minimum during the latter portion of the experimental trials. Mean h_{CO_2} in quail was within its conventionally assumed range in all but three 30 min intervals, and the expected lower limit was always within the 95% confidence interval of empirical data.

Discussion

Our data indicate that conventional methods of estimating thermal equivalents of gas exchange can produce large errors in calculations of metabolic power consumption. Two such methods are most commonly used by workers. Perhaps the most careful is to measure both O_2 consumption and CO_2 production, then estimate h_{CO_2} and h_{O_2} from RER and a linear interpolation between values for lipids and carbohydrates (Fig. 7). The error resulting from this approach can be approximated by comparison with our measured values.

In kangaroo rats, calculation of thermal equivalents based upon RER and linear interpolation produces absolute errors averaging 21% over the entire 17 h period, with the maximum mean error for a 30 min period reaching 38% (Fig. 7). In quail, absolute errors average 15% over the 17 h trial, with the maximum error for a 30 min period equaling 19%. In doves, absolute errors average 17% over the 17 h trial and the maximum 30 min error value equals 37%. These values may well underestimate actual errors, because data cannot be used when RER falls outside the expected range of 0.71–1.00, which occurred in both kangaroo rats and doves. Up to five individual kangaroo rats and up to eight individual doves were thus excluded from calculations of some 30 min mean errors.

A second approach, often used when only a single gas (either O_2 or CO_2) is measured, is to assume that an animal that has fasted for a long period is catabolizing only lipids used for energy storage. This, of course, greatly restricts the types of experimental manipulations that can be made. Even allowing the animal to fast 10 h and using only data collected over the final seven hours of experimental trials produced mean absolute errors of 11% (based on O_2 measurement) or 17% (based on CO_2 measurement) in kangaroo rats, 11% (O_2 measured) or 18% (CO_2 measured) in quail, and 8% (O_2) or 10% (CO_2) in doves (Fig. 8). Maximum errors for individual 30 min periods were much greater. Using CO_2 measurements, these errors reached 17% (doves) to 27% (kangaroo rats). Using O_2 measurements, mean maximum errors reached 14% (doves) to 16% (kangaroo rats).

Our values for the thermal equivalents of respiratory gas exchange differ so frequently from those conventionally assumed as to raise the question of inaccuracies in our analyses. There are three classes of possible errors in our measurements: those arising from respirometry, those from direct calorimetry, and those from changes in body temperature. As noted above, our measurements of respiratory gas exchange are accurate within 0.4%. Errors

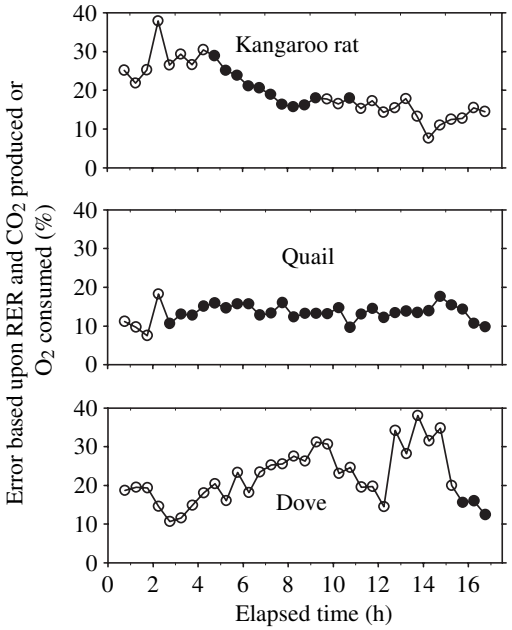

Fig. 7. Mean absolute error in estimates of the thermal equivalent of either CO_2 production or O_2 consumption produced by assuming that protein is conserved and using the respiratory exchange ratio to estimate the fractional content of lipids and carbohydrates in the catabolic substrate. Filled circles indicate N=10. Unfilled circles indicate that sample size was reduced below 10 because RER values outside of the range of 0.71–1.00 did not allow estimation of thermal equivalents for some individuals.

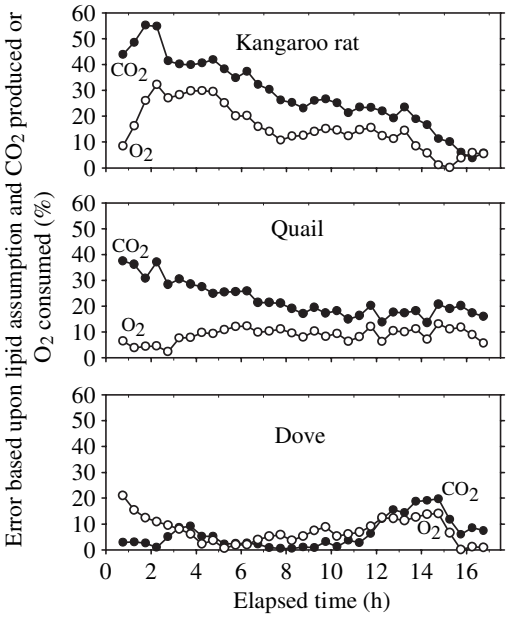

Fig. 8. Mean absolute error in estimates of the thermal equivalent of CO_2 production and O_2 consumption by measuring either gas and assuming that the animal is catabolizing lipids. N=10 for each species.

arising from direct calorimetry also are quite unlikely to explain our results. Because the range of thermal equivalent values observed is both significantly above and below conventional expectations, it is difficult to imagine an error in instrument calibration that could bring one set of values within the expected range and not drive another set farther outside of this range. For example, values for h_{O2} in kangaroo rats fall both within and substantially below the expected range (Fig. 5). If the unexpectedly low values resulted from calibration errors, then correcting this would simultaneously shift values that currently fall within the expected range to levels substantially *above* conventional expectations. Values for quail and doves would also be elevated substantially and would often exceed the conventional maximum. Given this and the linearity of our calibration (r^2=0.998), it seems inordinately improbable that the patterns we observe result from calibration errors.

Finally, our calculations assume that changes in body heat storage are insignificant. If body temperature increases, for example, then some metabolically produced heat is stored and not sensed by the calorimeter. This would lead to an underestimate of the thermal equivalents of gas exchange. Conversely, releases of heat due to decreases in body temperature would produce overestimates of h_{O_2} and h_{CO_2}. Ideally, such changes would be quantified by measuring average whole-body temperature and multiplying that by the specific heat capacity of the animal. Technical difficulties associated with measuring body temperature inside the sealed and submerged calorimeter prevented us from routinely doing this, but available evidence argues strongly against the importance of such effects. For example, we did succeed in measuring body temperatures of quail during four trials. These birds were part of a larger on-going experiment exploring effects of variation in diet, and only one of these animals is incorporated in the current data set. Body temperature, measured by indwelling thermocouples positioned both subcutaneously and intra-abdominally, exhibited net changes of only 0.3–1.6°C over the entire 17 h period. Over 30 min periods, an individual's maximum temperature change was 0.3–0.9°C. Such changes are much smaller than those required to produce the effects we observed. We estimate this by calculating body heat capacity as the product of body mass and the typical specific heat capacity of animals (3.47 J deg.$^{-1}$; Blaxter, 1989). This allows calculation of the body temperature change required to alter heat stores sufficiently to make values for thermal equivalents of ventilatory gases conform with those calculated on the basis of RER. Such calculations reveal that bringing our mean data into agreement with expected values for kangaroo rats requires net body temperature changes of 34°C (h_{O_2}) and 38°C (h_{CO_2}) over the 17 h period. In quail, 18°C (h_{O_2}) and 20°C (h_{CO_2}) average net changes are required and net changes of 16°C (h_{O_2}) and 20°C (h_{CO_2}) are necessary in doves. Shifts in body heat storage were thus excluded as an appreciable source of error. In doves, however, bringing observed values into conformance with expected values required the birds to decrease body temperature (T_b) 4°C–6°C during the first half of experimental trials then increase T_b

8–9°C during the latter half of trials. It therefore is possible that shifts in T_b may partially account for the effects observed.

Our results thus raise serious concerns regarding existing data (both modern and historical) that underlie much of our understanding of animal energetics. Errors in estimates of h_{CO_2} and h_{O_2} translate into identical fractional errors in estimates of metabolic power consumption. If patterns such as those we have observed occur in other species and situations, then differences between the actual thermal equivalents of respiratory gas exchange and those conventionally assumed by workers can produce major errors in estimates of metabolic power consumption. Such errors are on a par with differences that are used to support conclusions regarding animal energy usage in many studies. Four examples of such conclusions for birds are as follows. (1) Daily energy expenditure varies 3–26% over the annual cycle (Dawson and O'Connor, 1996); (2) resting metabolism averages 24% higher during the active phase of the daily cycle than during the rest phase (Aschoff and Pohl, 1970); (3) 20–50% of daily energy expenditure in passerine birds is devoted to thermoregulation (Dawson and O'Connor, 1996); (4) energy costs of embryonic development are about 30% higher in precocial species than in altricial species (Vleck and Vleck, 1987). Although we do not assert that our data invalidate these specific conclusions, this potential obviously exists.

What are the physiological origins of the discrepancies we observe between actual metabolic heat production and that estimated using indirect calorimetry, and how might the accuracy of respirometric estimates be improved? Candidate explanations for the discrepancies lie in the extensive series of assumptions that subsume the implicit view that animal respiratory and energy metabolism can be usefully described by a relatively constrained set of catabolic processes, such as oxidizing lipids completely to CO_2 and H_2O. For example, one complication is that respirometry can estimate only aerobic heat production whereas direct calorimetry measures the sum of both aerobic and anaerobic catabolism. This might provide a partial explanation for those cases in which the thermal equivalents of gas exchange are unexpectedly high. Accounting for anaerobic heat production would only exacerbate discrepancies in cases in which thermal equivalents are unexpectedly low, however. Additional complications might include, at a minimum, storage of carbon dioxide in the form of bicarbonate (e.g. Harrison, 1989; Harrison and Kennedy, 1994), consumption of oxygen by processes that release little heat (e.g. formation of oxygen radicals; Fridovich, 1998), and biochemical synthesis. For example, synthesis of tripalmitin from glucose is characterized by a respiratory quotient of 2.75 and thermal equivalents of h_{CO_2}=7.8 kJ l^{-1} and h_{O_2}=21.5 kJ l^{-1} (Blaxter, 1989).

Unfortunately, our knowledge of the physiological patterns and processes involved is rudimentary. Such knowledge would be less vital if variance remained low and discrepancies between actual and respirometrically estimated energy expenditure were predictable in direction and magnitude. In that case, errors in indirect calorimetry might be readily corrected. This is not possible with extant data, however. In doves, for example, variability in thermal equivalents was high and the coefficient of variance (=100×S.D./mean) for h_{O_2} averaged 25% over the entire 17 h trial. The problems arising from such intraspecific variance combined with interspecific differences are magnified by our ignorance of how factors such as phylogeny, nutritional status and history, tissue synthesis, and energetic challenges alter the errors in respirometric estimates. We cannot, therefore, prescribe measures to minimize errors in indirect calorimetry. To increase the likelihood that respirometric data are maximally useful in the future, however, it clearly is prudent for workers to report as fully as possible data describing the metabolic status of animals during measurements (e.g. body mass changes, RER, nutritional history) and to control such variables to the maximum extent possible in experimental trials. The case remains, however, that respirometric data must be used cautiously until the nature and origin of the discrepancies we have observed are well understood, as it is clear that these indirect estimates of metabolic heat production can subsume errors that are disturbingly large and of a magnitude sufficient to call into question generalizations regarding patterns of energy use in many studies.

List of symbols

$c_{p,dry}$	specific heat capacity of dry air
$c_{p,s}$	specific heat capacity of water vapor
F_C	fractional content of CO_2
F_O	fractional content of O_2
F_W	fractional content of H_2O
h	thermal equivalent
h_{CO_2}	thermal equivalent of carbon dioxide production
h_{EST}	estimated thermal equivalent
h_{O_2}	thermal equivalent of oxygen consumption
h_{OBS}	empirically observed thermal equivalent
Q_{conv}	convective heat flux
Q_{evap}	evaporative heat flux
R_B	rate of flow of air
RER	respiratory exchange ratio
$T_{a,in}$	temperature of influent air
$T_{a,out}$	temperature of effluent air
T_b	body temperature
$\dot{V}_{CO_2}$	rate of flow of CO_2
$\dot{V}_{O_2}$	rate of flow of O_2
$\dot{V}_{H_2O}$	rate of flow of H_2O
λ	latent heat of vaporization of water
ρ_{dry}	density of dry air
ρ_s	density of water vapor
$\rho_{v,in}$	vapor density of influent air
$\rho_{v,out}$	vapor density of effluent air

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