Validation of the Doubly Labeled Water Method in Bald Eagles (Haliaeetus leucocephalus) and a Comparison of Two Equations for the Calculation of Energy Expenditure

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ABSTRACT

We validated the doubly labeled water (DLW) technique for measurement of energy expenditure in bald eagles (Haliaeetus leucocephalus) in captivity by simultaneously measuring metabolizable energy intake in a feeding trial. We calculated CO2 production using two equations, one typically used by animal ecologists (the "one-pool" equation) and the other typically used by human nutritionists (the "two-pool" equation). Metabolizable energy intake, as determined by feeding trials, for two adult eagles eating rats averaged 1,160 ± 89 kJ d⁻¹ and for four nestlings eating fish averaged 2,124 ± 40 kJ d⁻¹. Energy expenditure measured from DLW turnover using the one-pool equation averaged 2.2% ± 7.1% higher than metabolizable energy intake measured by feeding trials (not significantly different, P > 0.50), but when the two-pool equation was used, energy expenditure measured with DLW averaged 17.7% ± 6.7% lower than metabolizable energy intake measured by feeding trials (significantly different, 0.025 < P < 0.05). Thus, the use of the DLW technique with CO2 production calculated by the one-pool equation was validated for bald eagles.

Introduction

Controversy over the correct calculation of CO2 production from turnover of doubly labeled water (DLW; Speakman 1993) prompted our validation study on bald eagles. Speakman (1993) reviewed animal and human validation trials with DLW and found that animal ecologists invariably calculate CO2 production using equations derived from Lifson and McClintock (1966) and Nagy (1980) (called "one-pool" equations in Speakman's [1993] terminology), but human nutritionists invariably use equations derived from Coward et al. (1985) or Schoeller et al. (1986; referred to as "two-pool" equations).

The major difference between the two groups of equations is in the use of the dilution spaces of the isotopes in the equations. In the one-pool equations, a single dilution space is calculated from the measurements of the oxygen isotope (usually 18O). In contrast, in the two-pool equations, separate dilution spaces for both oxygen and hydrogen isotopes are calculated from measurements of each isotope. The dilution spaces are then multiplied by the apparent fractional turnovers of the two isotopes.

Speakman (1993) found very little difference between metabolic rates calculated with these two methods for very small vertebrates. In fact, most ecological DLW measurements have been performed on small species (<2 kg; Speakman 1993), probably because the cost of the isotope 18O is high. But few recent animal studies of larger species (>5 kg) have used the one-pool equations without validation trials. Speakman (1993) suggests that the one-pool equations may overestimate energy expenditure in larger animals, owing to size-specific differences in rates of water flux and deuterium incorporation in fat synthesis.

By 1993, there had been only three validation studies in animals larger than 2 kg (Speakman 1993). Clearly, there is a need for a validation experiment in a large bird species such as the bald eagle (Haliaeetus leucocephalus).

Although the bald eagle is one of the largest wild bird species in North America, it does not quite approach animals nearing the mass of humans, the size that most concerned Speakman (1993). Nonetheless, most validations of DLW in birds have been done on much smaller species, and only one (Nielet et al. 1992) specifically addressed the question of the use of one-pool versus two-pool equations. This paucity of data, in combination with our recent DLW measurements in nestling bald eagles in the wild, led us to perform a validation trial for DLW in captive adult and nestling bald eagles.

Material and Methods

Adult Trial

In February 1992, the first validation trial was performed with two long-term captive adult female bald eagles at the Raptor
Figure 1. Body masses of four nesting eagles and two adult eagles used in the validation trials. Bird number and sex are identified.

Center, at the University of Minnesota at Minneapolis St. Paul. The birds had been previously housed with approximately six other eagles in a large outdoor flight pen at the Raptor Center and were allowed 1 d for acclimation to individual indoor cages, where the feeding trials were conducted.

Each bird was weighed on a large infant scale (±10 g; Toledo model 1072), and a background blood sample (approx. 1 mL) was taken from the brachial vein with a plastic 5-mL syringe tipped with a 20-gauge needle. The DLW dose for each eagle was calculated and weighed in a glass Hamilton syringe on a portable field balance (A & D, model EK-120A, ±0.01 g). The solution (0.35 g DLW kg\(^{-1}\) body mass; 0.23 g 99% D\(_2\)O kg\(^{-1}\) and 0.12 g 97% H\(_2\)O\(^{18}\)O kg\(^{-1}\)) was injected into the pectoralis muscle. The birds were placed in individual indoor cages. To determine equilibration time, we removed birds from the cages and took 1-mL blood samples at 2 h, 4 h, and 6 h after injection. After the 6-h sample, the feeding trial was begun.

After 3 d, 4 d, and 7 d, we removed the birds from their cages, weighed them, and took a 1-mL brachial blood sample from each. Each blood sample was centrifuged immediately, and the plasma was drawn off, flame-sealed into 75-μL capillary tubes, and refrigerated for later analysis.

For the simultaneous feeding trial, the birds were housed separately in indoor cages measuring approximately 50 × 102 × 91 cm. Cages contained only perches and were lined with heavy transparent plastic that covered the cage floor and extended halfway up the cage walls. The photoperiod was 12L : 12D (light, 0730–1930 hours). At 1600–1700 hours daily, each bird was given one frozen lab rat; the rats' heads, tails, and guts were removed before weighing (range, 120–305 ± 5 g wet) and feeding. Three sample rats were similarly prepared and frozen for later analysis.

Both eagles consumed all rats entirely, except on the first day of the trial. Neither bird gained or lost mass during the trial (Fig. 1). Hence we considered metabolizable energy intake to be equivalent to energy expenditure.

Excreta and pellets produced were collected on plastic linings, which were changed on day 3 and day 4. After removal, the plastic sheets were carefully folded and frozen. Frozen excreta was scraped from the plastic and stored in specimen cups in the freezer until drying. All pellets were removed and frozen separately.

Two sample rats were homogenized by autoclaving and then grinding in a Waring blender. The homogenates, the remaining rat, the excreta, and the pellets were dried at 50°C. Energy content of the food, excreta, and pellets was measured on a Phillipson microbomb calorimeter (Gentry Instruments). Excreta and pellet samples for each bird were pooled over the entire feeding trial for energy analysis. At least two replicates were run on each sample, and coefficients of variation averaged 1.1%.

Dilution samples of the DLW solution were made by mixing a weighed dose of DLW in distilled water (Table 1). Plasma samples and dilution samples were analyzed by isotope-ratio mass spectrometry at the University of Wisconsin—Madison Department of Chemistry. Samples were run in duplicate, with additional samples run if the coefficient of variation was greater than 5%. In a few cases, obvious outlier replicates were deleted if there were four or more replicates run. Average coefficients of variation for initial and final samples were 0.5% (for \(^{18}\)O) and 1.8% (D), and final samples were well elevated above background levels (Table 1). Isotope ratios were measured as differences (δD or δ\(^{18}\)O) from the Vienna Standard Mean Ocean Water (VSMOW) in parts per thousand (‰), using 2,005 parts per million \(^{18}\)O and 155.8 parts per million deuterium as the best estimates of VSMOW (Giovinatti 1978).

Nestling Trial

A second similar validation trial was conducted with two male and two female nesting bald eagles on June 10–14, 1994, at the Raptor Center at the University of Minnesota. The nestlings, age 51–56 d on June 10, were removed from their nests in north-central Wisconsin on June 6–9, 1994, held in temporary facilities in Minocqua, Wisconsin, and then transported by truck to the Raptor Center on June 9. None of the nestlings were siblings. Procedures for DLW were similar to those described for the adults, except that the dose was 0.28 g DLW kg\(^{-1}\) body mass (0.09 g D\(_2\)O kg\(^{-1}\) and 0.19 g H\(_2\)O\(^{18}\)O kg\(^{-1}\)) and was injected into the brachial vein. An equilibrium period of 2 h was allowed, on the basis of the results of the adult trial, and final blood samples were collected 3.98–3.99 d after the trial began.

Nestlings were weighed at 0800–1000 hours on June 10, 1994, before the first feeding of that day. DLW trials were begun at 1215–1249 hours, and the feeding trial was begun at 1550–1635 hours on June 10. Cages and plastic linings were the same as those described for the adult trial. Photoperiod was 14L : 10D (light, 0700–2100 hours). Eagles diet consisted of frozen wild-caught bullheads (Acteora spp.) ranging from approximately 150 to 400 g. The fins were removed from each
Validation of the Doubly Labeled Water Method in Eagles

The birds were fed 240–340 g wet weight twice per day (at approx. 0900 and 1600 hours), with each meal consisting of one or two bullheads. Eagles generally consumed all they were given, except for a few meals when fish heads and other small pieces were rejected. Uneaten food was retrieved at the next meal and refrozen for later analysis. We manipulated the amount offered at each meal, depending on the mass consumed in the previous meal, to keep the eagles' body masses constant. Seven sample bullheads were weighed and frozen for later analysis. Plastic cage linings were changed on day 2 and day 3. Uneaten food still on the plastic was separated and frozen. Excreta on plastic was frozen, scraped, and stored as in the adult trial. No pellets were produced by the nestlings.

On June 11–14, the nestlings were weighed daily between 1300 and 1600 hours, after their first feeding of the day. Although early morning weighing would have been preferable, logistics at the study facility prevented such timing. No birds gained mass during the trial (Fig. 1).

Three sample fish were dried in an oven at 50°C to measure water content of the food. Three sample fish were homogenized while still frozen in a blender and then ground in a Wiley mill, freeze-dried, and analyzed for energy content. Excreta samples were pooled over the entire trial. Uneaten food was classified into one of five categories (whole fish heads, partial heads, upper skulls only, heads and vertebrae, and all other bones), and two representative samples of each category were analyzed. Other uneaten food samples within each category were assumed to have similar energy density. Leftover food was analyzed in this manner because individual nestlings tended to leave different parts of the fish as remains. Coefficients of variation for energy content averaged 2.5% for replicates.

Dilution samples of the DLW solution were made by mixing a weighed dose of DLW in distilled water (Table 1). Plasma samples and dilution samples were analyzed by isotope-ratio mass spectrometry at Metabolic Solutions (Merrimack, N.H.). Samples were run in duplicate, with additional samples run if the coefficient of variation was greater than 5%. In a few cases (for D), outlier replicates were deleted if there were four or more replicates run. Average coefficients of variation of initial and final samples were 0.4% (18O) and 1.5% (D), and final samples were well above background levels (Table 1). The isotope ratio measurements were reported as differences from the VSMOW and so used in the calculations.

Calculations

For both trials, the apparent assimilable mass coefficient, AMC* (apparent because uncorrected for endogenous loss), was calculated as follows:

\[ AMC^* = \frac{(I - (E + p))/I}{U} \]

where I is food intake (g dry matter d⁻¹), E is excreta produced
(g dry matter d\(^{-1}\)), and \(p\) is pellets produced (g dry matter d\(^{-1}\)), with \(p = 0\) for nestlings. The apparent metabolizable energy coefficient, MEC\(^{p}\), was calculated as follows:

\[
\text{MEC}^p = \frac{|IK_p - (EK_p + pK_p)|}{IK_p}
\]

where \(K_p\) is the energy content of the food consumed in kg g\(^{-1}\) dry weight, \(K_i\) is the energy content of the excreta produced in kg g\(^{-1}\) dry weight, and \(K_p\) is the energy content of the pellets in kg g\(^{-1}\) dry weight.

For both one-pool and two-pool calculations, total body water (TBW, in milliliters) was estimated by \(^{18}\)O dilution space. The \(^{18}\)O dilution space, \(N_\text{O}\) (in milliliters), was calculated as follows:

\[
N_\text{O} = (O_{\text{in}} - O_{\text{out}})(V_{\text{ave}}V_{\text{DLW}})/(V_{\text{ave}}(O_\text{in} - O_\text{out}))
\]

where \(O_{\text{in}}\) is the concentration of \(^{18}\)O in the dilution sample (8\(^{18}\)O in \(^{\%}\) relative to VSMOW), \(O_{\text{out}}\) is the concentration of \(^{18}\)O in the distilled water used to dilute the injection solution in the dilution sample (8\(^{18}\)O in \(^{\%}\) relative to VSMOW), \(V_{\text{ave}}\) is the volume of distilled water used in the dilution sample (milliliters), \(V_{\text{DLW}}\) is the volume of the DLW injection solution used in the dilution sample (milliliters), and \(V_{\text{out}}\) is the volume of DLW solution injected into a bird (milliliters). \(V_{\text{ave}}\) and \(V_{\text{DLW}}\) were calculated from masses divided by the density of the solution; densities of the solutions were calculated from reference values and concentrations. The terms \(O_\text{in}\) and \(O_\text{out}\) are the concentrations of \(^{18}\)O in the bird’s initial and background blood samples, respectively (8\(^{18}\)O in \(^{\%}\) relative to VSMOW).

For adults and nestlings, since body masses were constant, we assumed TBWs were also constant throughout the trial.

CO\(_2\) production (\(r_{\text{CO}_2}\), in mL d\(^{-1}\)) was first calculated by the one-pool method (Nagy 1980), reexpressed in terms of mL d\(^{-1}\):

\[
r_{\text{CO}_2} = 622.3N_\text{O}(k_\theta - k_d)
\]

The apparent isotope turnover rates (\(k_\theta\) and \(k_d\), in d\(^{-1}\), for \(^{18}\)O and D, respectively) were calculated as follows:

\[
k_\theta = \frac{\ln(O_\text{in} - O_\theta) - \ln(O_\text{in} - O_\text{out})}{t_\theta}
\]

and

\[
k_d = \frac{\ln(D_\text{in} - D_\theta) - \ln(D_\text{in} - D_\text{out})}{t_d}
\]

where \(O_\theta\), \(O_\text{out}\), \(D_\theta\), and \(D_\text{out}\) are concentrations of \(^{18}\)O and D in the bird’s background, initial, and final blood samples (8\(^{18}\)O in \(^{\%}\) relative to VSMOW), and \(t\) is the length of the DLW trial (in days).

CO\(_2\) production was also calculated using the dilution spaces of both isotopes (two-pool method). Dilution space for D was calculated as follows:

\[
N_\text{D} = (D_{\text{in}} - D_{\text{out}})(V_{\text{ave}}V_{\text{DLW}})/(V_{\text{ave}}(D_\text{in} - D_\text{out}))
\]

where \(N_\text{D}\) is the dilution space for D (in milliliters), \(D_{\text{in}}\) is the concentration of D in the dilution sample (8D in \(^{\%}\) relative to VSMOW), and \(D_{\text{out}}\) is the concentration of D in the distilled water used to dilute the injection solution in the dilution sample (8D in \(^{\%}\) relative to VSMOW).

Rate of CO\(_2\) production (in mol d\(^{-1}\)) was calculated as suggested by Speakman (1993):

\[
r_{\text{CO}_2} = [(N_\text{O}/18.02)/2.08]((k_\theta - R_{\text{aqua}}k_\theta) - 0.0246k_d)
\]

where \(N_\text{O}\) was divided by 18.02 to convert milliliters to moles.

The term \(r_\theta\) is the rate of water loss, which is fractionated and is estimated as:

\[
r_\theta = 1.05(N_\text{O}/18.02)(k_\theta - R_{\text{aqua}}k_\theta)
\]

\(R_{\text{aqua}}\) is the mean dilution space ratio for all birds in the study, where \(R_{\text{aqua}}\) is calculated as follows:

\[
R_{\text{aqua}} = N_\text{D}/N_\text{O}
\]

Both calculated rates of CO\(_2\) production were converted to kJ d\(^{-1}\) using the relationships 22.400 mL CO\(_2\), mol\(^{-1}\) CO\(_2\), and 25.7 J mL\(^{-1}\) CO\(_2\) for a proteinaceous food source (Ricklefs 1974).

Energy intakes and expenditures are presented as mean ± SEM and were compared by t-tests. Differences in means for the apparent metabolizable energy coefficient and the apparent assimilable mass coefficient were tested by t-test (SISTAT; Wilkinson 1988).

**Results**

**Feeding Trials**

Rates of metabolizable energy intake in captivity (sometimes called Metabolized Energy\(_{\text{ave}}\) or Existence Metabolism by other investigators) for the adult and nestling eagles as determined by the feeding trials averaged 2,124 ± 40 kJ d\(^{-1}\) for nestlings and 1,160 ± 89 kJ d\(^{-1}\) for adults. However, the mean apparent assimilable mass coefficient and mean apparent metabolizable energy coefficient for the two diets did not vary significantly (apparent assimilable mass coefficient, 0.68 ± 0.01 and 0.67 ± 0.00 for fish and cleaned rats, respectively, \(P = 0.74\); apparent metabolizable energy coefficient, 0.82 ± 0.01 and 0.84 ± 0.01 for fish and cleaned rats, respectively, \(P = 0.12\).
Table 2: Validation feeding trials and DLW measurements in adult and nesting bald eagles at the Raptor Center, 1992 and 1994

<table>
<thead>
<tr>
<th>Bird No.</th>
<th>Sex</th>
<th>Age (d or yr)</th>
<th>Average Body Mass (g)</th>
<th>Intake (g d⁻¹)</th>
<th>Feeding Trial AMC b</th>
<th>Metabolizable Energy (kJ d⁻¹)</th>
<th>DLW Measurement: Energy Expenditure</th>
<th>One-Pool</th>
<th>Two-Pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>F</td>
<td>56 d</td>
<td>4,319</td>
<td>126.0</td>
<td>.65</td>
<td>.80</td>
<td>2.044</td>
<td>2,148</td>
<td>1,804</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>55 d</td>
<td>3,794</td>
<td>125.1</td>
<td>.70</td>
<td>.82</td>
<td>2.130</td>
<td>2,745</td>
<td>2,364</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>51 d</td>
<td>3,664</td>
<td>126.1</td>
<td>.66</td>
<td>.81</td>
<td>2.088</td>
<td>1,615</td>
<td>1,315</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>52 d</td>
<td>4,065</td>
<td>132.2</td>
<td>.70</td>
<td>.83</td>
<td>2.233</td>
<td>2,084</td>
<td>1,741</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>3,961</td>
<td>127.4</td>
<td>.68</td>
<td>.82</td>
<td>2.124</td>
<td>2,148</td>
<td>1,806</td>
</tr>
<tr>
<td>Adult L</td>
<td>F</td>
<td>5+ yr</td>
<td>4,660</td>
<td>57.8</td>
<td>.67</td>
<td>.84</td>
<td>1.248</td>
<td>1,217</td>
<td>901</td>
</tr>
<tr>
<td>Adult S</td>
<td>F</td>
<td>5+ yr</td>
<td>4,910</td>
<td>49.8</td>
<td>.67</td>
<td>.83</td>
<td>1.071</td>
<td>1,188</td>
<td>870</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>4,785</td>
<td>53.8</td>
<td>.67</td>
<td>.84</td>
<td>1.160</td>
<td>1,203</td>
<td>886</td>
</tr>
</tbody>
</table>

Note: AMC = Apparent metabolizable coefficient. MECA = Metabolizable Energy Coefficient.

DLW Measurements

The rate of energy expenditure as determined by DLW and calculated by the one-pool technique averaged 2,148 ± 232 kJ d⁻¹ for the four nestlings and 1,203 ± 15 kJ d⁻¹ for the two adults (Table 2). For adult S, technical problems invalidated three ¹⁸O replicates of the final sample. For this sample, we sent the remaining plasma (a single sample) to Metabolic Solutions. Because the mass spectrometer at Metabolic Solutions had a slightly different calibration than that at the University of Wisconsin, we adjusted the ¹⁸O values as suggested by Hoefs (1987). All samples for this bird were recalculated as ¹⁸O in %o relative to distilled water from our laboratory, a sample of which had been analyzed on both mass spectrometers. Recalculated values are presented in Table 1 and used in calculations.

For the two-pool calculations, the mean dilution space ratio in a study is a critical variable, since a small analytical error can result in large variance in CO₂ production (Speakman 1993). Speakman (1993) further suggested that differences among individuals of a group tend to be analytical, not physiological. For these reasons, researchers working on humans have settled on a common mean dilution space ratio, which is used in most studies (1.03; Schoeller et al. 1986), but Speakman (1993) suggested that animals may differ from humans in dilution space ratio and thus mean dilution space ratio for a particular group of animals should be used in calculating CO₂ production. We had three independent measures of dilution space ratio in nesting bald eagles from three studies (the present study and two field studies which were analyzed separately; Dykstra 1993). The average of the three values was 1.02 ± 0.06. It seemed unlikely that nestlings drawn randomly from the same geographical area would have a different mean dilution space ratio, which should be a physiological trait, and more likely that the differences between studies related to errors in the analysis of the dilution samples. For this reason, we calculated the rate of energy expenditure as determined by DLW and calculated by the two-pool technique for chicks using the mean dilution space ratio (1.02, essentially the same as the value used for humans; Schoeller et al. 1986). For nestlings, the mean rate of energy expenditure as determined by DLW and calculated by the two-pool technique was 1,806 ± 215 kJ d⁻¹ (Table 2).

For adults, we had no other measure of dilution space ratio besides that in this trial, so we used this value, 1.11; the rate of energy expenditure as determined by DLW and calculated by the two-pool technique averaged 886 ± 16 kJ d⁻¹ (Table 2).

Comparisons

Comparison of the two types of DLW equations for nestlings indicated that the calculations using the one-pool equation were more similar to measurements of metabolizable energy intake measured by feeding trial than the two-pool values (Tables 2, 3). Mean error averaged +2.2% ± 7.1% in one-pool calculations and -17.7% ± 6.7% in two-pool calculations (Ta-
Table 3: Mean algebraic error (includes sign) of one-pool and two-pool equations

<table>
<thead>
<tr>
<th>Bird</th>
<th>Error (One-Pool Model) (%)</th>
<th>Error (Two-Pool Model) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>+5.1</td>
<td>-11.7</td>
</tr>
<tr>
<td>17</td>
<td>+28.9</td>
<td>+11.0</td>
</tr>
<tr>
<td>14</td>
<td>-22.7</td>
<td>-37.0</td>
</tr>
<tr>
<td>13</td>
<td>-6.7</td>
<td>-22.1</td>
</tr>
<tr>
<td>Adult L</td>
<td>-2.5</td>
<td>-27.8</td>
</tr>
<tr>
<td>Adult S</td>
<td>+10.9</td>
<td>-18.8</td>
</tr>
<tr>
<td>Mean</td>
<td>+2.2 ± 7.1</td>
<td>-17.7 ± 6.7</td>
</tr>
</tbody>
</table>

Note. Error is calculated as \((\text{ME}_{\text{exp}} - \text{ME}_{\text{end}})/\text{ME}_{\text{end}}\times 100\), where \(\text{ME}_{\text{exp}}\) is the rate of energy expenditure as determined by DLW and \(\text{ME}_{\text{end}}\) is the metabolizable energy intake in captivity as determined by feeding trial.

Feeding Trial

Rates of metabolizable energy intake measured by feeding trials in captivity for the two adult bald eagles were similar to rates measured by Stalnaker and Gessaman (1982) in adult bald eagles: 258 kJ kg\(^{-1}\) d\(^{-1}\) for a mammalian diet and 221 kJ kg\(^{-1}\) d\(^{-1}\) for a fish diet (1.235 and 1.057 kJ d\(^{-1}\) for an adult of mass 4,785 g) but lower than their values for eagles eating birds (388 kJ kg\(^{-1}\) d\(^{-1}\); Stalnaker and Gessaman 1982). Similarly, Kirkwood's allometric equation for maintenance rates of metabolizable energy intake in captivity predicted 1,274 kJ d\(^{-1}\) for an adult of 4,785 g (Kirkwood 1981).

Measured apparent metabolizable energy intake coefficient for a fish diet (0.82) was similar to published values. For a fish diet, the average apparent metabolizable energy intake coefficient in bald eagles at three ambient temperatures was 0.75 (Stalnaker and Gessaman 1982). The average apparent metabolizable energy intake coefficient of fish when fed to birds of many species is 0.77 or 0.78 (Castro et al. 1989; Karasov 1990; Bennett and Hart 1993). The measured apparent metabolizable energy intake coefficient and apparent assimilable mass coefficient for the rat diet were not directly comparable to published values, nor ecologically relevant, since rats were headless and gutted.

It is not unusual for nestlings to have higher rates of energy intake than adults of the same species. Collopy (1986) found that captive golden eagle (Aquila chrysaetos) nestlings at peak intake consumed 97% more than captive adults (Feverholt and Craighead 1958). Wild nestlings also consumed more than wild adults (estimated intake; Collopy 1984, 1986). However, in long-term measurements of energy intake, nestlings inevitably grow, and some of the excess energy must be devoted to growth.

In this study, we controlled nestling intake to maintain stable body masses, so net energy was not deposited in new tissues. Rather, the nestlings were simply more active than the captive adults. The adults used in the study were long-term captives (8–9 mo), accustomed to cages and handling, and were less active than the nestlings. Nestlings in cages seemed nearly as active as they are in nests in the wild (C. R. Dykstra, personal observation).

Nestling rates of metabolizable energy intake in captivity did not seem particularly high when compared with nestling field metabolic rates. Nestling mean rate of metabolizable energy intake in captivity was 10%–17% lower than mean field metabolic rate at two locations in Wisconsin (Dykstra 1995).

Comparison of Equations

The validation trial indicated that the one-pool equation estimated energy expenditure well, although individual errors were large (Table 3). Large individual errors are common in validation studies (Speakman and Racey 1988; Nolet et al. 1992; Speakman 1993), and the maximum errors in our study (~23 to +29%) fall within the typical range of errors (Speakman and Racey 1988).

The mean dilution space ratio has a tremendous influence on resulting calculated CO\(_2\) production (Schoeller et al. 1986; Speakman 1993). The mean dilution space ratio is troubling in the context of field studies, where a single dilution sample is usually analyzed one time. (Since all values in a single trial depend on one dilution sample, there is really only one value per trial.) A single unvalidated field study using the two-pool method could be at a risk for error due to an analytical error in measurement of the dilution sample. In our work, we were fortunate to have had three separate studies in which the dilution space ratio was independently measured for nestlings. In comparing our dilution space ratio to published values (Schoeller et al. 1986), it seems that our range of ratios between studies was somewhat larger than most. For adults, we had only one measured dilution space ratio; we had 1.02 for them as well, the rate of energy expenditure as determined by DLW and calculated by the two-pool technique would have been 1,068 kJ d\(^{-1}\) for adult L and 1,040 kJ d\(^{-1}\) for adult S. This substitution changed the results only slightly: for one bird, the two-pool calculation became minimally better than the one-pool calculation (adult S). For adult L, the one-pool calculation was still clearly more accurate (Table 2). Overall, the values calculated from the one-pool model were still a better
estimate of energy expenditure than values calculated from the two-pool model; however, the two-pool values would no longer differ significantly from the feeding trial results ($t = 1.92, 0.10 < P < 0.20$).

A major difference in the two equations is that the two-pool equation assumes no irreversible nonaqueous loss of $D$ (which is alternatively called subsidiary $D$ flux, or sequestering of $D$). In contrast, the one-pool equation allows for such flux, if the rate of $D$ loss from the nonaqueous (subsidiary) pool is equal to the rate of $D$ loss from the aqueous pool (Speakman 1987, 1990).

However, the irreversible nonaqueous loss of $D$ has no effect on the calculations if it is irreversibly lost with $^{18}O$ in the same ratio as that found in water (2 D : 1 $^{18}O$). If the isotopes are sequestered into body tissue at the same ratio as occurs in water, there is no effect on calculation of energy expenditure (Haggarty et al. 1991). Thus de novo synthesis of fat can have a large effect on the DLW calculations since $H$ : $O$ ratios are very different from that in water (Roberts 1989), but little differential sequestering of isotopes in protein and carbohydrates occurs (Haggarty et al. 1991).

Overall, in situations in which substantial differential incorporation of hydrogen into body components other than water occurs, the one-pool equations should theoretically be more accurate (Speakman 1990, 1993). In cases in which subsidiary hydrogen flux does not occur, the two-pool equations should be more accurate.

A validation study with 1.8-kg geese found that the one-pool equation overestimated CO$_2$ produced by 13.9%, while a two-pool equation overestimated it by only 0.9% (Nolet et al. 1992). The difference between the two estimates (13.9%) was fairly similar to the difference we found between our two estimates (19.9%), which was logical, since the species are roughly similar in size. However, Nolet et al. (1992) found that the two-pool equation better fit their respirometry data. They attributed this to the fact that geese were catabolizing fat (Nolet et al. 1992). Thus, there was no irreversible nonaqueous sequestering of $D$, so the one-pool model was predicted to overestimate CO$_2$ production, which it did.

The eagles in our study were apparently in steady state, and thus there was negligible net deposition or catabolism of fat. Although there were no body mass changes, the nestlings were growing feathers during the 4 d of the trial. Average eighth primary length increased by 3.0 ± 0.2 cm in 4 d, and presumably other feathers grew proportionately. No studies have examined sequestering of hydrogen or oxygen in growing feathers, but it is unlikely that $D$ from water could be differentially sequestered in the keratin of feathers. Feather protein is probably similar to muscle protein, which has a limited capacity to sequester $D$ (Haggarty et al. 1991).

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Literature Cited


