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Division of Comparative Physiology and Biochemistry, Society for Integrative and Comparative Biology

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Estimation of Daily Energy Expenditure from Heart Rate and Doubly Labeled Water in Exercising Geese

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Abstract

We investigated whether daily $\dot{V}O_2$ consumption could be predicted from heart rate (f_H) in five exercising barnacle geese (*Branta leucopsis*) and compared the accuracy of this method with that of the doubly labeled water (DLW) method. The regressions of $\dot{V}O_2$ on f_H , based on incremental speed tests, differed among individual birds. The O_2 pulse (i.e., $\dot{V}O_2/f_H$) progressively increased with exercise level from 0.22 mL O_2 heartbeat^{-1} during resting to an estimated 0.47 mL O_2 heartbeat^{-1} during flight. Daily $\dot{V}O_2$ was generally underestimated (-3.9%) by (individual) resting O_2 pulses but overestimated ($+8.4\%$) by linear regressions of $\dot{V}O_2$ on f_H . However, it was well predicted ($+0.8\%$) by the O_2 pulses appropriate for each exercise level. When using relationships derived from the group of birds, the estimations were generally improved (-3.3% for resting O_2 pulse, -0.03% for appropriate O_2 pulse) but poorer ($+13.6\%$) for the group linear regression. Some of these predictions were better than the estimation of daily CO_2 production ($\dot{V}CO_2$) by the two-compartment model of the DLW method (average algebraic error of $+0.9\%$). We conclude that f_H can be used to estimate daily energy expenditure in birds accurately provided that (1) its application is limited to the range of exercise levels in which f_H has been calibrated against $\dot{V}O_2$ and (2a) $\dot{V}O_2$ - f_H relationships are determined for each individual bird or (2b) the f_H measurements of several free-ranging birds are averaged. Heart rate can also be used to indicate within-day variation in energy expenditure.

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Introduction

The doubly labeled water (DLW) method and the time-energy budget (TEB) method are being widely employed as largely noninvasive techniques to measure energy expenditure in free-living animals. The DLW method yields an average value of the CO₂ production ($\dot{V}CO_2$) over one or more days (Lifson, Gordon, and McClintock 1955). It has been validated against more traditional methods (e.g., respirometry, food balance method), and DLW estimates were generally accurate (i.e., average absolute error less than 10%) (Nagy 1989; Roberts 1989; Tatner and Bryant 1989). It should be noted, however, that most validation studies have involved animals in resting conditions, whereas the energy expenditure of free-ranging birds is generally more than twice the resting level (see, e.g., Bryant and Tatner 1991).

The TEB method is very laborious, because the animal's behavior has to be continuously observed. Short-term changes in energy expenditure can be estimated, but TEB estimates appear to be accurate only when both metabolic costs of different behaviors and thermoregulatory requirements have been measured (Weathers et al. 1984; Nagy 1989).

In laboratory experiments with birds it has been noted that heart rate (f_H) increases either linearly (Bamford and Maloiy 1980; Woakes and Butler 1983; Hüppop 1987) or hyperbolically (Grubb 1982; Grubb, Jorgensen, and Conner 1983) with O₂ consumption ($\dot{V}O_2$). The regressions were reported to explain 32%–94% of the total variation. According to the Fick principle, $\dot{V}O_2$ is related to cardiac output as

$$\dot{V}O_2 = f_H \times SV \times (C_a - C_v)O_2, \quad (1)$$

where SV is cardiac stroke volume and $(C_a - C_v)O_2$ is the difference between arterial and mixed venous O₂ contents. In the birds studied, SV plays a minor role in increasing cardiac output (Bech and Nomoto 1982; Grubb 1982; Grubb et al. 1983). Thus, the variation unexplained by the regressions of f_H on $\dot{V}O_2$ is probably mainly due to changes in $(C_a - C_v)O_2$.

It is clear that f_H can potentially be used as an indicator of metabolism in free-living animals. Owen (1969) and Gessaman (1980) tested whether f_H could be used to estimate daily energy expenditure in teals (*Anas discors*) and kestrels (*Falco sparverius*). They concluded that energy expenditure was fairly well predicted in some individuals, but not in others. Indeed, different relationships between $\dot{V}O_2$ and f_H have been found not only among individuals, but also in the same individuals during different experimental

conditions (Woakes and Butler 1983) or seasons (Gessaman 1980) or after physical training (Butler and Turner 1988).

A recorder has been developed in which f_H is stored (Woakes 1992). An animal can be equipped with this device and recaptured after a few days or weeks, and the f_H can be retrieved from the solid-state memory. This makes it important to test in more detail to what extent f_H can be used to estimate energy expenditure in the field. In this study we initially determined the relationship between f_H and $\dot{V}O_2$ in resting, walking, and swimming barnacle geese. We then continuously monitored f_H , $\dot{V}O_2$, and $\dot{V}CO_2$ of the same geese for 2 d. The geese were exercising to simulate the daily energy expenditure of free-living birds and were losing body mass (M). Simultaneously, $\dot{V}CO_2$ was estimated by the DLW method. We investigated the relative accuracies of estimating daily $\dot{V}O_2$ from f_H and daily $\dot{V}CO_2$ from the DLW measurements.

Material and Methods

Birds

Five barnacle geese (*Branta leucopsis*) were trained for 3–4 mo prior to the experiments to build up physical condition and to get fully accustomed to the experimental surroundings. Training consisted of swimming or walking at previously determined medium speeds for 2–4 h twice a week. At the start of the experiments the geese varied in M from 1.65 to 2.01 kg (table 1).

Apparatus

At night the geese rested in a dark respirometer box (dimensions 0.55 m \times 0.35 m \times 0.35 m high) with a single mixing fan and a small window on top.

The birds swam on a flume (Armfield Technical Education) with a test section of 1.2 m \times 0.5 m \times 0.5 m deep. The flume was fitted with a turbulence grid. Water speed could be varied between 0 and 1 m s⁻¹ by a variable-speed motor (Prestibloc G200M, Moteurs Leroy-Somer) and was measured to an accuracy of 0.02 m s⁻¹ by a Braystoke current flow meter (BFM002, Valeport Developments). Gas exchange was measured in an open-circuit Plexiglas respirometer box (dimensions 0.65 m \times 0.4 m \times 0.28 m high) containing two mixing fans. Flexible polyethylene sheets placed on the front and rear lower edges provided an airtight seal against the moving water, while the side edges projected 5 cm below the water surface.

TABLE 1

Initial body mass (M), length of sternum (L_s), condition index (M/L_s³), isotope dilution spaces of ¹⁸O and ²H at zero time (N_{O0} and N_{D0}), and dilution space fraction (F_O and F_D) in five barnacle geese

Bird	M (kg)	L _s (cm)	Condition		F _O	N _{D0} (mol)	F _D
			Index (kg dm ⁻³)	N _{O0} (mol)			
V	1.74	13.1	.775	52.9	.55 ^a	56.6	.59 ^a
L	1.65	13.6	.654	54.9	.60 ^a	58.2	.64 ^a
P	1.77	14.1	.631	64.0	.65	65.3	.67
Y	2.01	13.4	.837	58.0	.52 ^a	62.4	.56 ^a
D	1.74	13.7	.676	54.6	.57	58.6	.61
Mean . . .	1.78	13.6	.715	56.9	.58	60.2	.61
SE06	.17	.039	2.0	.02	1.6	.02

^a Estimated from the condition index (see text).

The geese walked on a level treadmill (Powerjog EV2, Sports Engineering). The belt speed could be varied between 0 and 5 m s⁻¹. The respirometer box (see above) was placed on top of a wooden frame (0.65 m × 0.3 m × 0.15 m high) mounted over the treadmill. Because of the extra volume of the frame, four fans were used to ensure rapid mixture of air. The four lower edges of the frame were made airtight by brush-type draft excluders that were in close contact with the smooth belt.

Respirometry

Air was drawn through the respirometer at 10–15 L min⁻¹ when the birds were resting and at 15–20 L min⁻¹ when they were active, these flows being selected in order to keep the concentration of CO₂ in the box below 0.5%. Flow rate was measured by variable-area flowmeters (1100 series, Fisher Controls). The sample air flow (1 L min⁻¹) was dried by a column of silica gel, and CO₂ was absorbed in front of the O₂ analyzer by a column of soda lime. Samples of air flowed separately through an S-3A stabilized zirconia cell O₂ analyzer (Ametek/Thermox Instruments) and a SS100 infrared CO₂ analyzer (Analytical Development Co.). According to the specifications of the manufacturers, the accuracy of the instruments was within ±0.02% O₂ and ±0.03% CO₂, respectively. Inlet air (10 min) and outlet air (20 min)

were sampled alternately by automatically switching a rotary valve with a microcomputer (BBC-B, Acorn). Oxygen and CO₂ concentrations were sampled every second at the built-in analog-to-digital (A/D) converter of the microcomputer, averaged over 1 min, and stored on disk. To allow flushing of the sampling lines, the first 5 min of sampling inlet or outlet air were excluded from the analysis. Carbon dioxide was absorbed from the outlet air by a column of soda lime in front of the main air circuit flowmeter (but beyond the branching to the gas analyzers).

Oxygen consumption was calculated according to formula (IA4) of Depocas and Hart (1957) after the appropriate modification of inlet air flow (\dot{V}_I) to CO₂-free inlet air flow (\dot{V}_I') following the procedure of Hill (1972):

$$\dot{V}_{O_2} = \dot{V}_I' F'_{IO_2} - (\dot{V}_I' - \dot{V}_{O_2}) F'_{EO_2} \quad (2a)$$

and

$$\dot{V}_I' = \dot{V}_E' + \dot{V}_{O_2}, \quad (2b)$$

where F'_{IO_2} and F'_{EO_2} are the O₂ concentrations of CO₂-free samples of inlet and outlet air, respectively, and \dot{V}_E' is the measured CO₂-free outlet airflow. Substitution gives

$$\dot{V}_{O_2} = \dot{V}_E' \frac{F'_{IO_2} - F'_{EO_2}}{1 - F'_{IO_2}}. \quad (2c)$$

Carbon dioxide production was calculated as

$$\dot{V}_{CO_2} = \dot{V}_E F_{ECO_2} - \dot{V}_I F_{ICO_2}, \quad (3a)$$

$$\dot{V}_E = \dot{V}_E' / (1 - F_{ECO_2}), \quad (3b)$$

$$\dot{V}_I = \dot{V}_I' / (1 - F_{ICO_2}), \quad (3c)$$

and

$$\dot{V}_I' = \dot{V}_E' + \dot{V}_{O_2}, \quad (3d)$$

where \dot{V}_E is the outlet air flow, and F_{ICO_2} and F_{ECO_2} are the CO₂ concentrations of samples of inlet and outlet air, respectively. Substitution gives

$$\dot{V}_{CO_2} = \frac{\dot{V}_E' F_{ECO_2}}{(1 - F_{ECO_2})} - \frac{(\dot{V}_E' + \dot{V}_{O_2})}{(1 - F_{ICO_2})} F_{ICO_2}. \quad (3e)$$

Gas flows were corrected to STPD (represented by \dot{V}).

Relative humidity of inlet and outlet air was measured with a humidity meter (Humicap 14). Evaporative water loss (\dot{V}_{H_2O}) was crudely estimated as the difference between $\dot{V}E'$ and $\dot{V}I'$ minus $\dot{V}O_2$ (see Appendix). Evaporative water loss may have been overestimated because of the evaporation of water from the feces.

The gas analyzers and the A/D converter of the microcomputer were calibrated by flowing two gas mixtures of known N₂, O₂, and CO₂ composition through the sample air circuit. The respirometer was tested for leaks and adequate mixing by infusing N₂ gas at a known rate. Overall accuracy of the system was assessed by infusing a known N₂, O₂, and CO₂ gas mixture at a known rate (0.67 L min⁻¹) into the box with gas mixing pumps (M301a-F and SA27/3-F, Wösthoff oHG), thus simulating the presence of a bird. The \dot{V}_{CO_2} and $\dot{V}O_2$ were measured with algebraic errors of -0.3% and -0.6% and absolute errors of 2.4% and 4.2% of the actual values, respectively. However, when the system was tested with the respirometer positioned on the flume, \dot{V}_{CO_2} measurements were inaccurate because of the CO₂ absorption by the water. Thus, CO₂ production of birds on the flume was not determined.

Heart Rate Telemetry

A pulse-interval-modulated (PIM) radiotransmitter was implanted into the abdominal cavity of a bird, at least 2 wk prior to an experiment. Anesthesia was induced with 2% halothane in a 3:1 air:O₂ mixture and maintained with 1%–1.5% halothane supplemented by local anesthesia (2% wt/vol Xylocaine with adrenaline, 1:80,000). The signal from the transmitter was passed from a receiver (Sharp) to a PIM decoder, which extracted the ECG. The decoded signal was passed to an instantaneous heart rate meter, the output of which was recorded on a thermal pen recorder (Lectromed). The traces were digitized by a GTCO digitizing pad (Digipad 5) taking a sample of f_H every minute.

Relationships between $\dot{V}O_2$ and f_H

The relationships between $\dot{V}O_2$ and f_H were first determined. Resting gas exchange and f_H were measured by placing each bird overnight in the dark box (with the window sealed) after a 24-h fast. Ambient temperature (17°–20°C) was held within their thermoneutral zone (Calder and King 1974). These conditions were appropriate for measuring basal metabolic rate (BMR) as defined by Kleiber (1961). Minimum half-hourly values of \dot{V}_{CO_2} , $\dot{V}O_2$, and f_H were taken.

Gas exchange and f_H were also measured during incremental speed tests both while the birds were swimming on the flume and while they were walking on the treadmill. Each test lasted 4 h; after 1 h at zero speed (preexercise) the speed was progressively increased in half-hourly intervals by about 0.1 m s^{-1} . The \dot{V}_{O_2} , \dot{V}_{CO_2} (on the treadmill only), and f_H were measured during a period of steady state of at least 5 min. Limb frequency was determined by clocking 100 steps or strokes of the left leg.

In a regression analysis, \dot{V}_{O_2} was taken as the dependent and f_H as the independent variable, because the equations were to be used to predict \dot{V}_{O_2} from f_H .

Doubly Labeled Water Technique

A dose of about $0.30 \text{ mL H}_2^{18}\text{O}$ (90.02%) kg^{-1} and $0.15 \text{ mL } ^2\text{HHO}$ (99.8%) kg^{-1} was subcutaneously injected as a mixture. The syringe was weighed before and after the injection to determine the injection dose, but in three of the five experiments a drop appeared when the needle was pulled out, and the injection dose could not be precisely determined. The time until the isotopes were in complete equilibration with the body water was determined in two separate trials in which blood samples were taken every hour after injection. The abundances of both isotopes decreased after 4 h, and this was taken as the equilibration time. Blood samples (ca. $10 \mu\text{L}$) were taken by puncturing the wing or tibial veins. The glass microcapillaries were flame sealed and stored at 5°C .

Samples were processed in duplicate. For each glass microcapillary, water was extracted from the blood samples by vacuum distillation. The water was stored with a known volume of CO_2 at 25°C . After equilibration for 24 h the water and CO_2 were separated cryogenically in a vacuum. Hydrogen was extracted from the water fraction by uranium reduction at 800°C . The isotope ratios (R 's) in CO_2 and H_2 were measured by isotope ratio mass spectrometry. If duplicates differed by more than 5%, another sample was processed.

The isotope ratio measurements of $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ were reported as deviations from Vienna-standard mean ocean water (V-SMOW) in parts per thousand (delta per mil). These were converted back to R 's (Wong and Klein 1986), and from these to atom percent excess (APE) values (Schoeller et al. 1986b).

The disappearance rates (k) of ^{18}O and ^2H (k_O and k_D , respectively) were calculated by the two-sample method (see Speakman and Racey 1986):

$$k = \frac{\ln(\text{APE}_i) - \ln(\text{APE}_f)}{t_f - t_i}, \quad (4)$$

where t is the time in days after the injection and the subscripts i and f denote initial and final samples, respectively. For both isotopes, the theoretical APE value at injection (APE_0) was calculated by extrapolation to zero time (t_0):

$$\text{APE}_0 = \exp[k(t_i - t_0) + \ln(\text{APE}_i)]. \quad (5)$$

The R at zero time (R_0) was derived by summation with the background R (R_{bg}):

$$R_0 = R_{\text{bg}} + [(\text{APE}_0/100) / (1 - \text{APE}_0/100)]. \quad (6)$$

From this and the injection doses of heavy isotopes, ²H or ¹⁸O (“hdose”), and light isotopes, ¹H or ¹⁶O (“ldose”), the dilution space at zero time (N_0) was calculated (for both deuterium [N_D] and ¹⁸O [N_O]):

$$N_0 = R_{\text{bg}} \frac{\text{hdose} - \text{ldose} R_0}{R_0 - R_{\text{bg}}} + \frac{\text{hdose} - \text{ldose} R_0}{R_0 - R_{\text{bg}}} \quad (7)$$

(to express N_D in moles H₂O instead of moles H the above equation is divided by two).

In the three experiments in which some of the injection dose was lost, we had to estimate the dilution spaces. For this purpose we successfully injected five more geese and found negative relationships between the deuterium and ¹⁸O dilution space fractions (F_D and F_O) (i.e., the dilution space per unit of M) and a condition index, defined as M (kg) divided by the cube of the sternum length (L_s in decimeters):

$$F_O = 0.894 - 0.448 M L_s^{-3} \quad (8)$$

and

$$F_D = 0.918 - 0.430 M L_s^{-3}. \quad (9)$$

These linear regression equations ($r^2 = 0.79$ and 0.80 , respectively, both $P < 0.05$) were used to estimate the initial dilution spaces of the three experimental birds. We assumed a proportional change in dilution spaces with M in the course of the experiments (Roberts et al. 1986).

Carbon dioxide and total water efflux rates (r_{CO_2} and $r_{\text{H}_2\text{O}}$) were determined for the eight possible combinations of initial, final, and background duplicate blood samples. Efflux rates were calculated with the assumption that there is an exponential change of dilution spaces with rate k_{N} according to the hypothesis of Lifson and McClintock (1966):

$$k_{\text{N}} = \ln(N_{\text{f}}/N_{\text{i}})/(t_{\text{f}} - t_{\text{i}}). \quad (10)$$

Water efflux was calculated as

$$r_{\text{H}_2\text{O}} = \frac{N_{\text{Di}} k_{\text{N}} (k_{\text{D}} - k_{\text{N}}) (t_{\text{f}} - t_{\text{i}})}{(1 - N_{\text{Di}}/N_{\text{Df}}) [f_1 p + (1 - p)]}, \quad (11)$$

where f_1 , f_2 , and f_3 are the in vivo physical fractionation factors (Wong et al. 1988), and p is the proportion of water loss subjected to fractionation. Carbon dioxide efflux rate was calculated according to a one-compartment model, as

$$r_{\text{CO}_2} = \frac{N_{\text{oi}} k_{\text{N}} (k_0 - k_{\text{D}}) (t_{\text{f}} - t_{\text{i}})}{2 f_3 (1 - N_{\text{oi}}/N_{\text{of}})} - \frac{f_2 - f_1}{2 f_3} p r_{\text{H}_2\text{O}}, \quad (12)$$

and according to a two-compartment model, as

$$r_{\text{CO}_2} = \frac{k_{\text{N}} (t_{\text{f}} - t_{\text{i}})}{2 f_3} \left[\frac{N_{\text{oi}} k_0}{(1 - N_{\text{oi}}/N_{\text{of}})} - \frac{N_{\text{Di}} k_{\text{D}}}{(1 - N_{\text{Di}}/N_{\text{Df}})} \right] - \frac{f_2 - f_1}{2 f_3} p r_{\text{H}_2\text{O}}. \quad (13)$$

We checked whether p was close to 0.5—the value assumed by Lifson and McClintock (1966)—by comparing p with the quotient of $\dot{V}_{\text{H}_2\text{O}}$ (measured by respirometry converted to moles per day) and $r_{\text{H}_2\text{O}}$. (In the calculation of the latter, p is needed, and therefore some circularity is involved here, but this effect is small).

Experiments

The experiments lasted three consecutive days. The levels of \dot{V}_{O_2} , \dot{V}_{CO_2} , and $\dot{V}_{\text{H}_2\text{O}}$ were measured for at least 21 h d⁻¹ by respirometry. Heart rate was recorded continuously on the last 2 d. Blood samples were taken at the start and the end of the experiment, yielding averages of $r_{\text{H}_2\text{O}}$ and r_{CO_2} for the 3-d experiment.

At 1400 hours on day 0, the bird was weighed, blood samples were taken in order to measure R_{bg} , and the isotope mixture was injected. The bird was placed in a dark box, and the respirometry system was calibrated. At 1900 hours on day 0, the bird was weighed again, and initial blood samples were taken. The bird was subsequently placed in the resting respirometer box, and gas exchange measurements were started. For the next 3 h food, water, and light were supplied. Thereafter, food and water were removed and the bird was kept in darkness during the night. At 0900 hours on day 1, fresh food and water were offered and the respirometer system was recalibrated over 2 h, during which period gas exchange was not determined. Gas exchange measurements during the following 30 min were used to estimate gas exchange during calibration. Subsequently, the bird was mounted in the Plexiglas respirometer box, which was placed over the treadmill. The bird walked in half-hourly intervals at 0.44–0.88 m s⁻¹ for up to 6.5 h d⁻¹. The same procedure was repeated for another 2 d, during which heart rate was continuously recorded. Values of \dot{V}_{O_2} and f_H were averaged over 30-min periods. At 1900 hours on day 3, the bird was weighed, and final blood samples were taken.

Statistics

The O₂ consumption predicted from f_H (\hat{V}_{O_2}) was calculated from three different relationships between \dot{V}_{O_2} and f_H : (1) the O₂ pulse (i.e., the \dot{V}_{O_2} per heartbeat) during resting (ROP), (2) the linear regression of \dot{V}_{O_2} on f_H (LR) from the incremental speed tests (including resting), and (3) a combination in which the O₂ pulse appropriate for a given time of day and activity is used (AOP): the resting O₂ pulse (s) during the night, the pre-exercise O₂ pulse (s) while the birds were inactive during the day, and the linear regressions from the incremental speed tests (excluding resting) when the birds were active during the day. For each bird, its individual \dot{V}_{O_2} - f_H relationship and the average \dot{V}_{O_2} - f_H relationship of the group of birds were used to predict \hat{V}_{O_2} .

Algebraic errors were calculated as $(\dot{V}_{O_2} - \hat{V}_{O_2})/\hat{V}_{O_2} \times 100\%$ and $(r_{CO_2} - \hat{V}_{CO_2})/\hat{V}_{CO_2} \times 100\%$. Absolute values of these are called absolute errors. All results are given as mean \pm SE. Unless stated otherwise, $n = 5$. Differences between mean values were tested with paired t -tests. Correlations are described with the Pearson product-moment correlation coefficient r , and in regression analyses the coefficient of determination r^2 is given. Differences in slopes and intercepts were tested with Student's t statistics. Variation in errors was analyzed by ANOVA using a regression

approach. Two-tailed probability values P of less than 0.05 were regarded as significant.

Results

Heart Rate and $\dot{V}O_2$ during Resting and Incremental Speed Tests

The respiratory exchange ratios (R_E) of the fasting birds were rather low (0.67 ± 0.02). However, this is close to the predicted respiratory quotient (RQ) of 0.71 for fat metabolism (Schmidt-Nielsen 1983). Resting $\dot{V}O_2$ was 9.28 ± 0.44 mL O₂ min⁻¹ kg⁻¹. This is similar to the 9.27 mL O₂ min⁻¹ kg⁻¹ predicted by equation (5) of Aschoff and Pohl (1970) assuming fat metabolism with an energy equivalent of 19.7 J mL O₂⁻¹ (Schmidt-Nielsen 1983). The R_E was 0.77 ± 0.03 during the preexercise period and 0.76 ± 0.02 while the birds were walking. During walking, $\dot{V}O_2$ and f_H increased linearly with speed (fig. 1), as is the case in most species previously studied (Taylor, Heglund, and Maloiy 1982). During swimming, $\dot{V}O_2$ and f_H increased exponentially with speed (fig. 2), as has been reported for other species (Prange and Schmidt-Nielsen 1970; Woakes and Butler 1983; Videler and Nolet 1990). However, f_H differed much more between individuals than either $\dot{V}CO_2$ or $\dot{V}O_2$. Maximum $\dot{V}O_2$ during the incremental speed tests was 4.9 ± 0.1 times resting $\dot{V}O_2$, while maximum f_H was only 3.8 ± 0.4 times resting f_H (table 2). Limb frequency (f_L in hertz) increased linearly with both walking speed (m s⁻¹), as $f_L = 3.34$ speed + 1.09 ($r^2 = 0.70$, $n = 27$, $P < 0.0001$), and swimming speed, as $f_L = 4.46$ speed + 0.24 ($r^2 = 0.70$, $n = 28$, $P < 0.0001$).

The O₂ pulse during resting was 0.22 ± 0.01 mL O₂ heartbeat⁻¹ (table 2). The O₂ pulse during the preexercise period was 0.20 ± 0.02 mL O₂ heartbeat⁻¹ and was not significantly different from the resting O₂ pulse. During maximum sustainable exercise the O₂ pulse was 0.29 ± 0.02 mL O₂ heartbeat⁻¹ both while walking and while swimming, which is significantly higher than that during resting ($P < 0.05$) or preexercise ($P < 0.05$).

In most cases, the linear regression lines of $\dot{V}O_2$ of f_H of the data from the flume and treadmill separately were similar within birds (except in bird V, in which the regressions differed in intercept, and in bird D, in which the regressions differed in both slope and intercept). It was therefore decided to combine the data from the flume and treadmill tests for each individual. The resulting relationships between $\dot{V}O_2$ and f_H were essentially linear (fig. 3, top). The linear regressions of $\dot{V}O_2$ explained 88%–95% of the total variation. The slope (b) of the regression lines was larger than the ROP in four

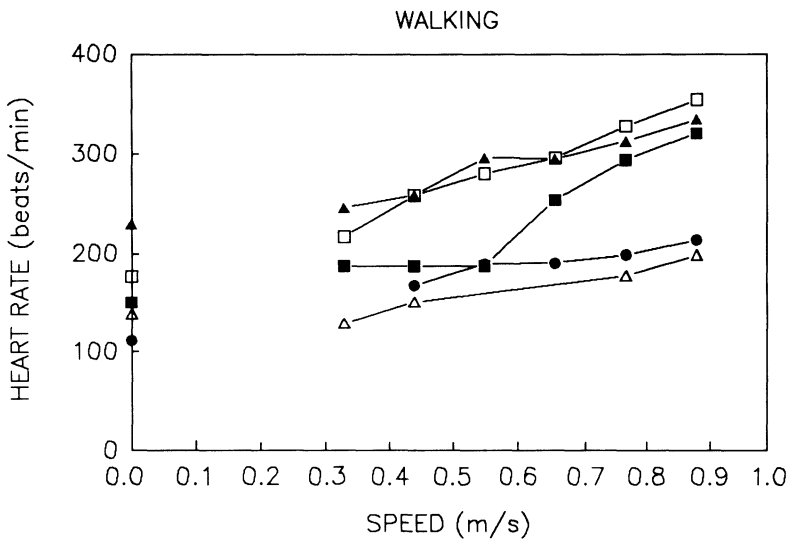
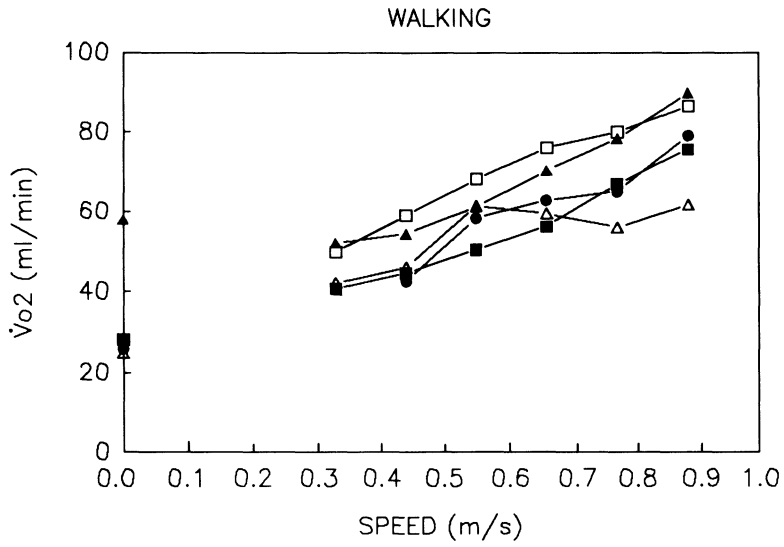


Fig. 1. Increase of $\dot{V}O_2$ (top) and f_H (bottom) with walking speed on a treadmill in five barnacle geese. Filled circle, goose V; open triangle, goose L; filled triangle, goose P; open square, goose Y; filled square; goose D.

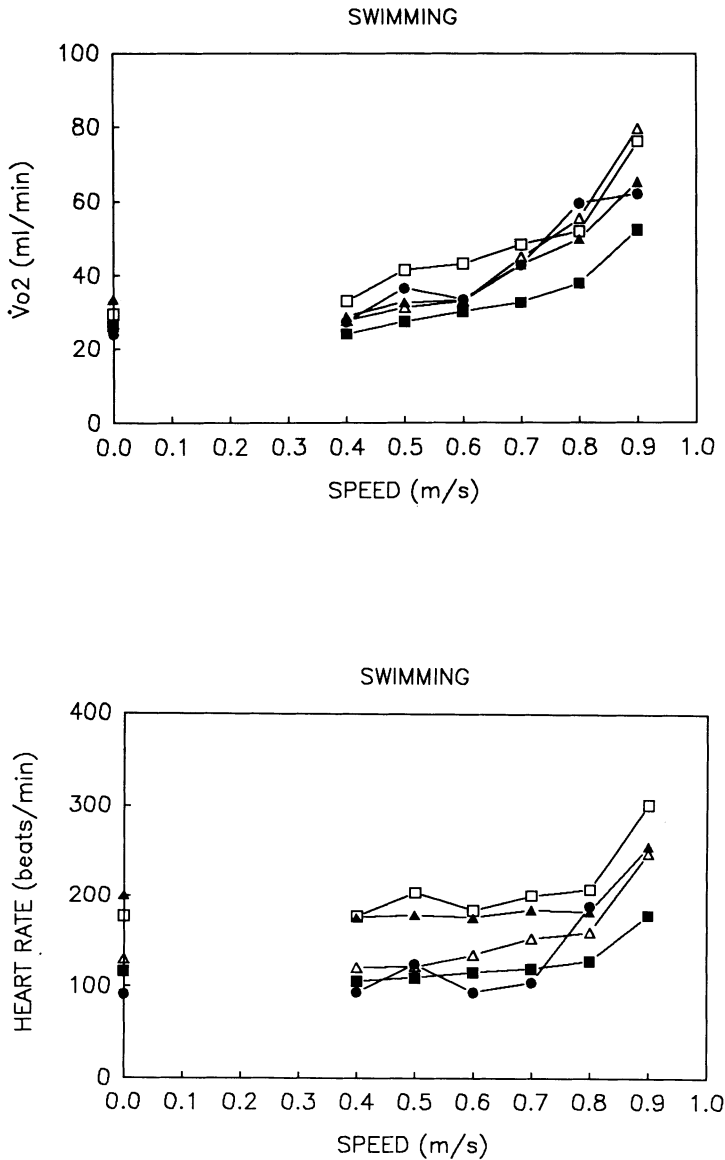


Fig. 2. Increase of \dot{V}_{O_2} (top) and f_H (bottom) with swimming speed in a flume in five barnacle geese. Symbols are as in fig. 1.

birds (V, L, P, and Y), and the intercept (a) was significantly less than zero in three birds (L, P, and Y) (table 3). This indicates that f_H did not rise directly in proportion to increases in \dot{V}_{O_2} during activity. Resting \dot{V}_{O_2} values were within the 95% confidence interval (CI) of the regression lines, but seven of the 10 preexercise \dot{V}_{O_2} values lay below it. Thus, although the O_2

TABLE 2
Oxygen consumption, f_H and O_2 pulse ($\dot{V}O_2/f_H$) of five barnacle geese in four conditions: fasting during the night ('resting'), prior to the incremental speed test ('preexercise'), and at the maximum sustainable swimming and walking speeds ($\dot{V}CO_2$ during resting is also given)

Bird	Resting				Preexercise				Swimming				Walking			
	$\dot{V}CO_2$ (mL min ⁻¹)	$\dot{V}O_2$ (mL min ⁻¹)	f_H (beats min ⁻¹)	O_2 Pulse (mL O_2 beat ⁻¹)	$\dot{V}O_2$ (mL min ⁻¹)	f_H (beats min ⁻¹)	O_2 Pulse (mL O_2 beat ⁻¹)	$\dot{V}O_2$ (mL min ⁻¹)	f_H (beats min ⁻¹)	O_2 Pulse (mL O_2 beat ⁻¹)	$\dot{V}O_2$ (mL min ⁻¹)	f_H (beats min ⁻¹)	O_2 Pulse (mL O_2 beat ⁻¹)	$\dot{V}O_2$ (mL min ⁻¹)	f_H (beats min ⁻¹)	O_2 Pulse (mL O_2 beat ⁻¹)
V	12.0	15.7	73	.22	26.0	112	.23	62.1	184	.34	79.0	214	.37	79.0	214	.37
L	10.7	16.9	72	.23	24.9	139	.18	79.6	249	.32	61.6	199	.31	61.6	199	.31
P	12.5	19.0	104	.18	58.0	230	.25	65.3	257	.25	89.9	335	.27	89.9	335	.27
Y	10.9	16.7	79	.21	28.8	177	.16	76.2	302	.25	86.5	354	.24	86.5	354	.24
D	10.3	16.0	66	.24	28.2	151	.19	52.5	179	.29	75.6	321	.24	75.6	321	.24
Mean	11.3	16.9	79	.22	33.2	162	.20	67.1	234	.29	78.5	285	.29	78.5	285	.29
SE	.4	.6	7	.01	6.2	20	.02	4.9	23	.02	4.9	32	.03	4.9	32	.03

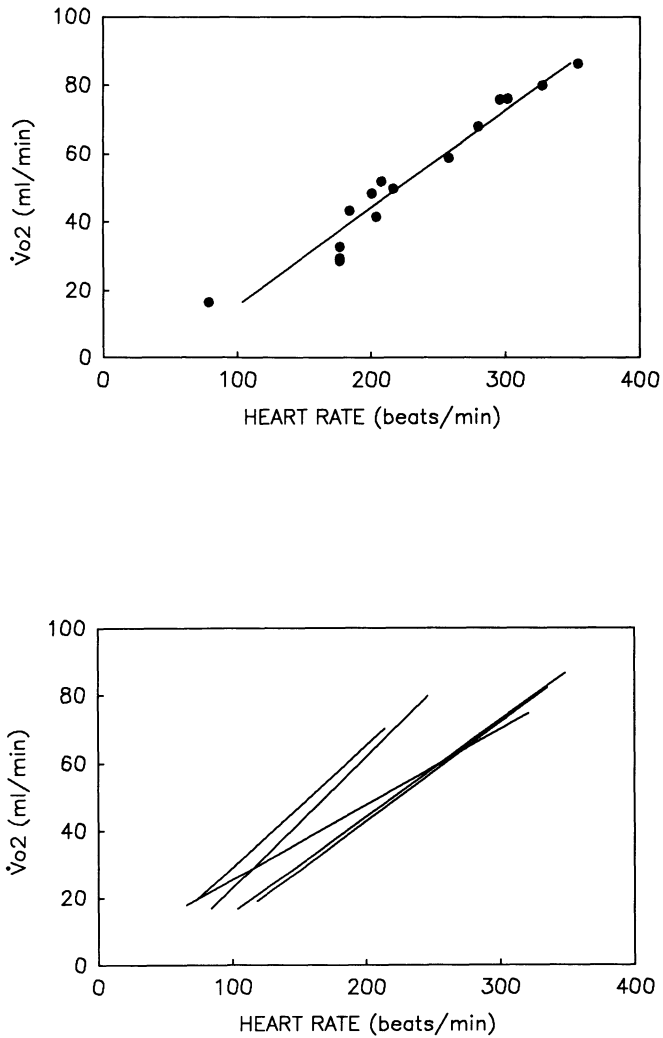


Fig. 3. Linear regressions of \dot{V}_{O_2} on f_H during resting conditions and incremental speed tests in barnacle goose Y (top) and in five barnacle geese (bottom).

pulses during preexercise did not differ significantly from the O_2 pulses during resting, they were lower than would be expected from the regressions of \dot{V}_{O_2} on f_H .

Each bird had a characteristic relationship between \dot{V}_{O_2} and f_H (fig. 3, bottom). A pairwise comparison of the linear regressions showed that either slope or intercept differed in eight of the 10 pairwise comparisons. Nevertheless, when all birds were pooled, the linear regression explained 79% of the variation in \dot{V}_{O_2} , but, in contrast to most of the individual linear

TABLE 3

Intercept (a) and slope (b) of linear regressions of $\dot{V}O_2$ (mL min⁻¹) on f_H (beats min⁻¹) in five barnacle geese during incremental speed tests swimming on a flume and walking on a treadmill (the linear regression of $\dot{V}O_2$ on f_H using the pooled data of all five birds is given under "group")

Bird	<i>n</i>	<i>a</i>	95% CI	<i>b</i>	95% CI	<i>r</i> ²
V	13	-7.1	-20 to +5	.36	.28-.44	.89
L	13	-15.8	-31 to -1	.39	.29-.48	.88
P	15	-15.5	-30 to -1	.29	.23-.35	.89
Y	15	-12.9	-22 to -3	.29	.25-.33	.95
D	15	+3.2	-4 to +10	.22	.19-.26	.93
Group . . .	71	+2.2	-4 to +8	.24	.21-.27	.79

regressions, its slope was not different from the resting O₂ pulse and its intercept did not differ from zero (table 3).

Daily $\dot{V}O_2$ and Change in M

Daily $\dot{V}O_2$ ranged from 1.6 to 2.1 times resting $\dot{V}O_2$, with a mean of 1.9 ± 0.1 . The geese lost $10.7\% \pm 0.5\%$ of *M*. Body mass decreased curvilinearly but not strictly exponentially with time: the specific daily change in *M* (i.e., the change in *M* per unit of *M*) sharply decreased with time (fig. 4). However, average *M* calculated under the assumption of an exponential change overestimated actual average *M* by only 1.7%. The specific daily change in *M* was correlated with the exercise level expressed as the quotient of daily $\dot{V}O_2$ and resting $\dot{V}O_2$ ($r = 0.99$, $P < 0.002$); in other words, the geese lost relatively more mass when exercise was more strenuous.

Doubly Labeled Water Estimates of Carbon Dioxide Production

The *R*_{bg}'s ($n = 10$) were 0.01559 ± 0.00016 atom percent for ²H and 0.20123 ± 0.00083 atom percent for ¹⁸O. During the experiment, the APE values in the blood samples dropped on average from 0.02328 to 0.01626 (²H), and from 0.04696 to 0.02603 (¹⁸O).

The quotient of \dot{V}_{H_2O} and r_{H_2O} was not significantly different from 0.5 (table 4). Because of the inaccuracies involved in estimating *p*, we used $p = 0.5$.

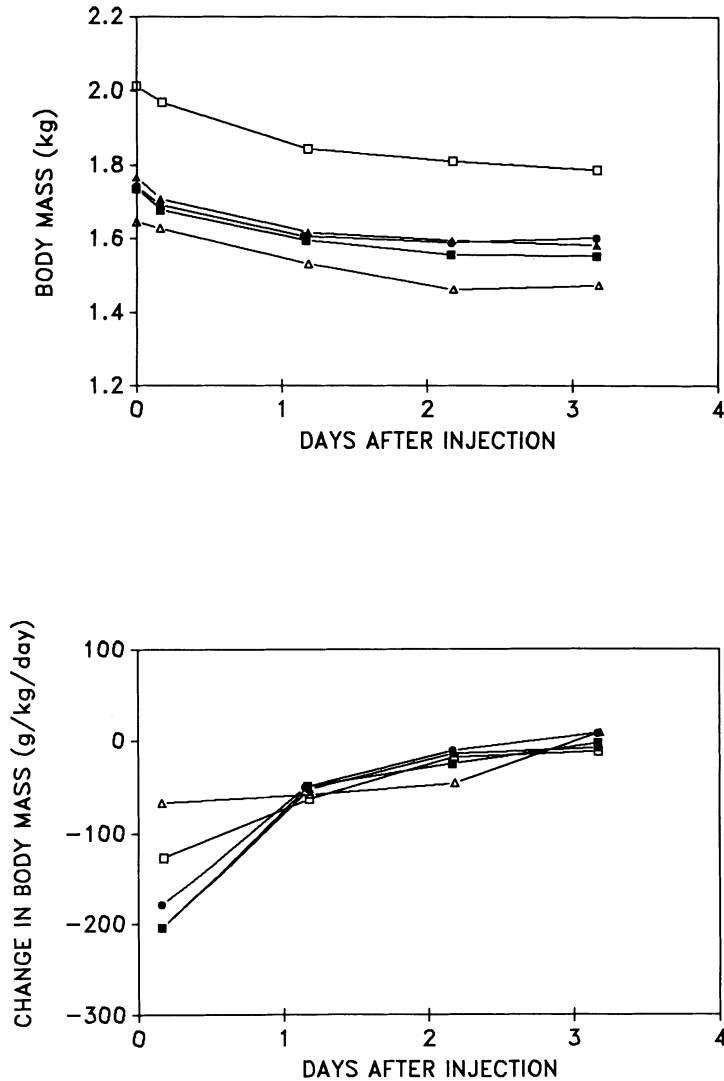


Fig. 4. Change in M (top) and mass-specific daily change in M during the experiments in five barnacle geese. Symbols are as in fig. 1.

The average k_o was 0.217 d^{-1} , and the average k_D was 0.137 d^{-1} (ratio $k_o/k_D = 1.58$), giving isotope half-lives of 3.66 ± 0.25 and $6.33 \pm 0.71 \text{ d}$, respectively. The average half-life of ^{18}O was significantly greater than predicted by allometric equations of Nagy (1983, eq. [2] from table 2: 2.42 d) and Tatner and Bryant (1989, eq. [10] modified for a 40% lower k_o in wintering birds: 2.23 d). These allometric equations are derived from measurements in small bird species (e.g., 12–410 g in Tatner and Bryant [1989]), and they might have little predictive power for larger birds. The duration

TABLE 4

Water loss measured by respirometry (\dot{V}_{H_2O}) and labeled water (r_{H_2O}) in five barnacle geese

Bird	\dot{V}_{H_2O} (mol d ⁻¹)	r_{H_2O} (mol d ⁻¹)	p^a
V	3.09	5.08	.61
L	3.29	10.06	.33
P	4.19	7.40	.57
Y	4.37	11.11	.39
D	3.89	8.65	.45
Mean . . .	3.77	8.46	.47
SE25	1.05	.05

^a Estimated as the quotient of \dot{V}_{H_2O} and r_{H_2O} .

of the experiments (3 d) was one half-life of ¹⁸O and 0.5 times the half-life of ²H, which is the minimum duration to obtain reliable results from the DLW measurements (see, e.g., Nagy 1983).

The DLW estimate of the one-compartment model overestimated the respirometric measurement of \dot{V}_{CO_2} by an average of 13.8% (table 5). Use of the two-compartment model reduced the average algebraic error to only +0.9% (fig. 5).

Predicting \dot{V}_{O_2} from f_H

When daily \dot{V}_{O_2} was predicted from the ROPs, \dot{V}_{O_2} was underestimated in most cases but the range of errors was small (table 6). The LRs, however, overestimated \dot{V}_{O_2} . When AOPs were used, daily \dot{V}_{O_2} was predicted within an algebraic error of 1%. The absolute error and the error ranges were smaller when individual instead of group relationships were used. Daily \dot{V}_{O_2} and \dot{V}_{O_2} were positively correlated, which indicates that relative differences between individuals were generally correctly predicted (fig. 6).

In order to determine whether daily \dot{V}_{O_2} of some birds was overestimated whereas that of others was underestimated, we predicted \dot{V}_{O_2} for each experimental day separately (i.e., $n = 10$, five birds \times 2 d). Indeed, differences in individuals alone explained as much as 81% (when individual AOP was used) or even 95% (when group AOP was used) of the total variation in errors (ANOVA).

Within days, half-hourly averages of \dot{V}_{O_2} and f_H were highly correlated in

TABLE 5

Comparison of daily CO_2 production measured by respirometry ($\dot{V}\text{CO}_2$) and the DLW method ($r\text{CO}_2$) in five exercising barnacle geese

Bird	$\dot{V}\text{CO}_2$ (mol d ⁻¹)	$r\text{CO}_2$ (mol d ⁻¹)	
		One- Compartment Model	Two- Compartment Model
V	1.19	1.32 ± .09	1.18 ± .09
L	1.52	1.33 ± .02	1.11 ± .02
P	1.75	2.04 ± .03	1.98 ± .05
Y	1.73	2.22 ± .06	1.91 ± .07
D	1.52	1.89 ± .03	1.64 ± .03
Algebraic error (%) ..		+13.8	+9
Absolute error (%) ..		18.9	11.8
Error range (%)		-13 to +28	-27 to +13
<i>r</i>81	.80

all five birds (table 7). However, $\dot{V}\text{O}_2$ predicted from AOPs correlated significantly better than f_{H} with $\dot{V}\text{O}_2$ in some cases (table 7).

Discussion

Daily $\dot{V}\text{O}_2$ and Change in *M*

The measured average daily $\dot{V}\text{O}_2$ of about $2 \times \text{BMR}$ is similar to the energy expenditure of free-living birds during their nonreproductive phase of the year. During breeding, and especially the nestling-rearing phase, the field energy expenditure is usually higher, between 3 and $4 \times \text{BMR}$ (and in some cases as high as $7 \times \text{BMR}$) (Bryant and Tatner 1991).

The geese lost *M* while exercising. Le Maho et al. (1981) found that, in geese in which *M* decreased from 6.25 to 5.65 kg, total body water decreased only from 3.9 to 3.8 L, but their birds were totally fasted and were drinking ad lib. Because our geese had limited access to both food and water, it is assumed that the proportion of total body water remained constant. As noted by Le Maho et al. (1981) the mass-specific change in *M* decreased with

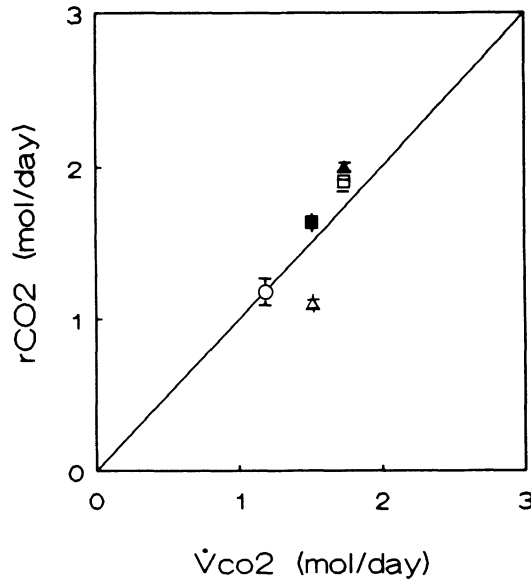


Fig. 5. Daily CO₂ production measured by respirometry (\dot{V}_{CO_2}) vs. CO₂ production calculated from simultaneous DLW measurements (r_{CO_2}) in five exercising barnacle geese. Values of r_{CO_2} were calculated according to a two-compartment model. Symbols are as in fig. 1.

time, but the assumption of an exponential change in M (i.e., pool size) did not seriously affect the DLW estimate.

Doubly Labeled Water: One- versus Two-Compartment Models

Most DLW validation studies produced good agreement of average values but considerable ranges of individual errors (Nagy 1989; Roberts 1989; Tanner and Bryant 1989). In our exercising and mass-losing geese, the average accuracy of the two-compartment model was within 1%, but the range of individual errors was also large. The one-compartment model overestimated \dot{V}_{CO_2} .

The two-compartment model was developed when it was recognized that the N_D is generally larger than the N_O (Lifson and McClintock 1966). The N_O is nearly equal to the total body water pool, whereas ²H is rapidly incorporated into a nonaqueous pool (mainly fat) after injection of the isotopes (Lifson et al. 1955). Roberts et al. concluded that if loss of hydrogen from the nonaqueous pool via nonaqueous routes is small, the one-compartment model will underestimate r_{H_2O} and, in consequence, overestimate r_{CO_2} (Roberts, Coward, and Lucas 1987). In the present study, the geese

TABLE 6
Comparison of daily O₂ consumption measured by respirometry ($\dot{V}O_2$) and predicted from f_H ($\hat{V}O_2$) in five exercising barnacle geese

Bird	$\dot{V}O_2$ (mol d ⁻¹)									
	$\dot{V}O_2$ (mol d ⁻¹)					$\hat{V}O_2$ (mol d ⁻¹)				
	Individual Relationships					Group Relationships				
	ROP	LR	AOP	LR	AOP	ROP	LR	ROP	LR	AOP
V	1.63	1.42	1.92	1.56	1.43	1.72	1.46			
L	2.23	2.16	2.56	2.29	1.99	2.34	2.07			
P	2.11	2.05	2.27	2.32	2.42	2.82	2.50			
Y	2.25	2.38	2.39	2.40	2.44	2.83	2.52			
D	2.11	1.98	2.02	1.89	1.76	2.36	1.84			
Algebraic error (%)		-3.9	+8.4	+8	-3.3	+13.6	-0.3			
Absolute error (%)		6.2	10.2	6.9	12.5	14.1	12.2			
Error range (%)		-13 to +6	-4 to +18	-10 to +10	-17 to +15	-1 to +34	-13 to +19			
r		.97***	.79	.90*	.77	.77	.79			

Note. $\hat{V}O_2$ was calculated with three different methods: resting oxygen pulse (ROP), linear regression (LR), and a combination of appropriate oxygen pulse (AOP), using individual and group relationships (see text).

* $P < 0.05$.

*** $P < 0.001$.

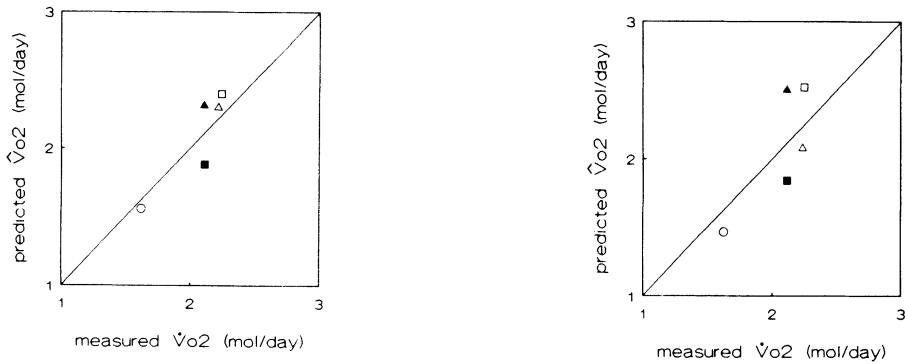


Fig. 6. Daily $\dot{V}O_2$ measured by respirometry vs. daily $\hat{V}O_2$ predicted from f_H in five exercising barnacle geese. Both individual (left) and group (right) $\dot{V}O_2$ - f_H relationships were used to predict $\hat{V}O_2$, from AOPs (see text). Symbols are as in fig. 1.

were catabolizing fat, and most of the ²H—incorporated in the fat in the first hours after injection—was, therefore, probably lost via the body water pool, which makes the two-compartment model more appropriate. Changes in background ²H levels due to fat catabolism are not considered here,

TABLE 7

Correlation between half-hourly-measured $\dot{V}O_2$ and f_H , and between half-hourly-measured $\dot{V}O_2$ and predicted $\hat{V}O_2$ O₂ consumption in five exercising barnacle geese during 2 d

Bird	n	f_H	$\hat{V}O_2$	
			Individual	Group
V . . .	85	.83	.95***	.90*
L . . .	85	.84	.90	.92*
P . . .	81	.78	.74	.89*
Y . . .	86	.92	.95	.96*
D . . .	87	.94	.92	.96

Note. $\hat{V}O_2$ was predicted from f_H from individual and group relationships (AOP; see text). All product-moment correlation coefficients are significantly different from zero ($P < 0.001$). Asterisks indicate significance levels of differences between $\dot{V}O_2$ - f_H and $\dot{V}O_2$ - $\hat{V}O_2$ correlation coefficients.

* One-tailed $P < 0.05$.

*** One-tailed $P < 0.001$.

although the DLW method is moderately sensitive to these changes (Schoeller, Leitch, and Brown 1986*a*; Haggarty and McGaw 1988).

Heart Rate as a Predictor of $\dot{V}O_2$

Our method of predicting daily $\dot{V}O_2$ from f_H (the “heart rate method”) gave good results (errors less than 10%) under certain conditions.

The use of ROPs (i.e., $\dot{V}O_2/f_H$) to predict $\dot{V}O_2$ from f_H implicitly assumes that f_H increases in direct proportion to increases in $\dot{V}O_2$ (i.e., when represented graphically, the relationship goes through the origin). However, in three of the five birds used in the present study, the linear regressions obtained from the incremental speed tests had a 's significantly less than zero. The nonproportionality may result from the fact that, in exercising birds, increased O_2 delivery is achieved by increases in both f_H and $(C_a - C_v)O_2$ (Butler, West, and Jones 1977; Bech and Nomoto 1982; Grubb 1982; Grubb et al. 1983). The result is that, when the ROPs are used, measured f_H underestimates $\dot{V}O_2$ whenever it is above the resting level (fig. 7).

Conversely, the use of LRs from the incremental speed tests will tend to overestimate average daily $\dot{V}O_2$ if arousal, with little or no increased activity (i.e., additional f_H ; Blix, Strømme, and Ursin 1974), occurs during pre- and post exercise (fig. 7). Because the O_2 pulse changed with time of day and activity, application of the AOPs produced the best prediction of daily $\dot{V}O_2$ in terms of algebraic error. The absolute error was, however, as large as that of ROP (table 6).

In two barnacle geese, Butler and Woakes (1980) measured a mean flight f_H of 512 beats min^{-1} . If f_H is taken to be proportional to $M^{-0.28}$ (Grubb 1983), a flight f_H of 497 beats min^{-1} is predicted for a goose of 1.78 kg (the mean M of the experimental birds). From the mean linear regression equation (table 3), this gives a $\dot{V}O_2$ of 122 mL min^{-1} . However, from the allometric equations of Masman and Klaassen (1987; non-wind-tunnel studies) and Butler (1991; wind-tunnel studies), $\dot{V}O_2$'s during flight of a 1.78-kg bird would be 261 and 226 mL min^{-1} , respectively. The flight O_2 pulses would be 0.53 and 0.45, respectively, in agreement with the 0.47 $\text{mL } O_2$ heartbeat $^{-1}$ predicted by the interspecific equation of Berger and Hart (1974). This implies that, during flight, $\dot{V}O_2$ increases by a greater proportion than f_H , compared with the situation during swimming and walking. Thus, the daily energy expenditure of birds that spend a large part of their day flying can be estimated from f_H only when the $\dot{V}O_2$ - f_H relationship during flight is also determined (fig. 7).

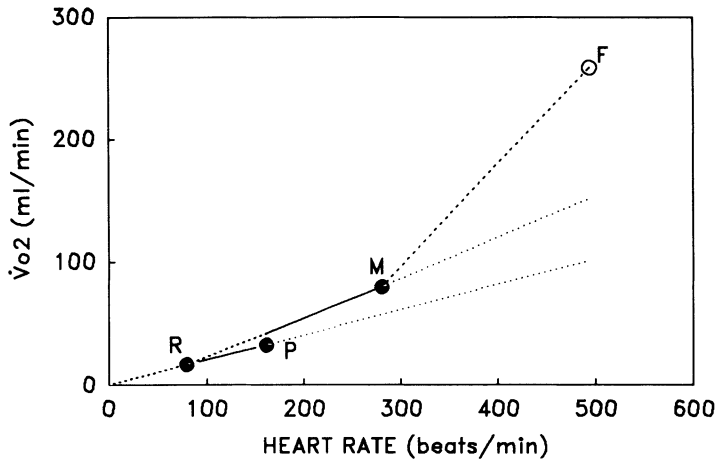


Fig. 7. Graphic representation of the relationship between \dot{V}_{O_2} and f_H using ROPs (R) and using the LR from incremental speed tests with intercepts less than zero (R to M). If the former are used to predict daily \dot{V}_{O_2} from measured f_H during exercise, \dot{V}_{O_2} is underestimated. If the latter is used to predict daily \dot{V}_{O_2} that includes periods of arousal with little or no activity (P, for "preexercise"), \dot{V}_{O_2} will be overestimated. The O_2 pulse during maximum sustainable walking or swimming speed (M) is smaller than the O_2 pulse during flight (F). Daily \dot{V}_{O_2} of birds that spend part of their day flying will be underestimated by measured f_H if the \dot{V}_{O_2} - f_H relationship during flight is not known, and ROPs or the LR are used instead.

It is a common phenomenon that the \dot{V}_{O_2} - f_H relationships differ among individuals (Owen 1969; Gessaman 1980). Indeed, we showed that, if f_H is used to estimate daily energy expenditure of individual birds, the \dot{V}_{O_2} - f_H relationship should be determined for each bird involved. Daily \dot{V}_{O_2} of each individual was poorly estimated by group \dot{V}_{O_2} - f_H relationships, but group \dot{V}_{O_2} - f_H relationships gave reasonable estimates of average daily \dot{V}_{O_2} . Therefore, if group \dot{V}_{O_2} - f_H relationships are used, the measurements of several birds should be averaged. Because in our study the group \dot{V}_{O_2} - f_H relationships were derived from the same birds that were used in the experiments, it is possible that individual errors could be even larger when these group \dot{V}_{O_2} - f_H relationships are applied to predict daily \dot{V}_{O_2} of other barnacle geese. However, Bevan, Keijer, and Butler (1991) showed that in tufted ducks (*Aythya fuligula*) a \dot{V}_{O_2} - f_H relationship determined in one group could be used to predict—from f_H —average \dot{V}_{O_2} in another group. This is the case even if the conditions under which the data were deter-

mined—for example, environmental temperature—are different (Bevan and Butler 1992).

Half-hourly f_H and \dot{V}_{O_2} proved to be good indicators of half-hourly \dot{V}_{O_2} , and, therefore, f_H can be used to indicate within-day variation in energy expenditure (i.e., to determine time budgets) of free-living birds. Thus, measuring f_H is valuable even when no relationships between f_H and \dot{V}_{O_2} are determined, especially if it is measured in conjunction with the DLW method, which yields an average value of the energy expenditure. Nagy (1989) also advocates the simultaneous use of several methods in field bioenergetics studies.

We conclude that, if the birds spend little time per day (less than a few percent) flying (e.g., during incubation and moult), the heart rate method (using AOPs) will yield reliable estimates of daily \dot{V}_{O_2} of individual barnacle geese (if individual \dot{V}_{O_2} - f_H relationships are used) or of a group of barnacle geese (if the measurements of several birds are averaged). Its accuracy compares favorably with that of the DLW method and the TEB method. Of course, determination of the AOPs is laborious, especially when each bird has to be calibrated, and use of ROPs is often more practical. However, as discussed above, use of ROP has its limitations and will generally underestimate daily \dot{V}_{O_2} . Even without determination of \dot{V}_{O_2} - f_H relationships, f_H can be used to indicate within-day variation in energy expenditure in free-living birds.

Acknowledgments

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Appendix Calculation of Evaporative Water Loss

Symbols used are

\dot{V}	Gas flows at room temperature and barometric pressure
\dot{V}	Gas flows corrected to STPD (except \dot{V}_{H_2O} , which is corrected to STP)
\dot{V}'_E	CO ₂ -Free outlet air flow
\dot{V}_{O_2}	O ₂ Consumption
\dot{V}'_I	CO ₂ -Free inlet air flow
\dot{V}_{H_2O}	Evaporative water loss

T_R	Room temperature
T_0	Standard temperature
P_B	Barometric pressure
P_V	Vapor pressure in saturated conditions
P_0	Standard pressure
RH_E	Relative humidity of outlet air
RH_I	Relative humidity of inlet air
F'_{EO_2}	O ₂ Concentration of outlet air
F'_{IO_2}	O ₂ Concentration of inlet air

In air entering and leaving a respirometer box encompassing a bird that is consuming O₂ and producing water vapor the following relationship holds (CO₂ being absorbed):

$$\dot{V}'_I - \dot{V}_{O_2} = \dot{V}'_E - \dot{V}_{H_2O},$$

where \dot{V}'_E is measured,

$$\dot{V}_{O_2} = \dot{V}'_{O_2} \frac{T_R}{T_0} \frac{P_0}{(P_B - P_V RH_E)},$$

and

$$\dot{V}'_I = \dot{V}'_I \frac{T_R}{T_0} \frac{P_0}{(P_B - P_V RH_I)},$$

with

$$\dot{V}'_E = \dot{V}'_E \frac{T_0}{T_R} \frac{(P_B - P_V RH_E)}{P_0},$$

$$\dot{V}_{O_2} = \dot{V}'_E \frac{F'_{IO_2} - F'_{EO_2}}{1 - F'_{IO_2}},$$

and

$$\dot{V}'_I = \dot{V}'_E + \dot{V}_{O_2}.$$

Substitution and rearranging gives

$$\dot{V}_{H_2O} = \dot{V}'_E \left(1 + \frac{F'_{IO_2} - F'_{EO_2}}{1 - F'_{IO_2}} \right) \left(1 - \frac{P_B - P_V RH_E}{P_B - P_V RH_I} \right)$$

and

$$\dot{V}_{H_2O} = \dot{V}'_E \frac{T_0}{T_R} \frac{P_B}{P_0}.$$

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