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Accounting for body condition improves allometric estimates of resting metabolic rates in fasting king penguins, *Aptenodytes patagonicus*

Abstract We describe a method that allows prediction of resting metabolic rate (RMR, ml $O_2 \cdot min^{-1}$) in adult male and female king penguins on shore by measuring body mass (M_b) and the length of the foot, flipper and beak. This method is accurate, underestimating measured RMR ($n = 114$) by 4% in a data set consisting of 44 birds (33 males and 11 females). Measurement error was unbiased with respect to fasting duration and can therefore estimate RMR during any stage of fasting. This new method provides significant cost and logistical savings when estimating RMR during fieldwork, allowing RMR of a large number of birds to be measured quickly. These findings suggest the possibility that the use of M_b and morphometrics will allow development of general and specific equations to estimate RMR in other species.

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Introduction

It is well known that body mass (M_b) correlates with resting metabolic rate (RMR) both within (e.g. Daan et al. 1989) and between species (e.g. Kleiber 1932; Daan et al. 1989). However, intra-specific variation in M_b may be associated with differences in nutritional status as well as structural size of the body, defined by Piersma (1988) as the size of the essential framework (also called the nutrient reserve-independent size). For species that undergo prolonged fasts such as the king penguin, *Aptenodytes patagonicus*, changes in M_b caused by differences in nutritional status can be substantial (approaching 30% during a 28 days fast, Froget et al. 2001; Fahlman et al. 2004). The change in M_b is due to mobilization of subcutaneous fat deposits, which are the major site of body reserves (~47% of total M_b, Cherel et al. 1994b). In addition, fasting leads to disproportionate changes in the size of metabolically active organs (Cherel et al. 1994a). For example, the masses of pectoral muscle and liver are reduced about 45% while the heart and hind limb muscles are reduced by approximately 25% (Cherel et al. 1994a). Therefore, the difference between M_b and structural size of the body represents an index of the nutritional state, because it is mainly represented by the parts of the body mobilized and used during fasting.

The disproportional changes in metabolically active and inactive organs and tissues suggest that M_b as a proxy for size in these animals may lead to substantial errors. Furthermore, given that M_b in king penguins varies by around 30% and RMR by about 48% during a normal fast (Fahlman et al. 2004, 2005), standard allometric equations cannot adequately predict RMR in king penguins. One way around this problem is to measure structural size and shape of the animal, which in addition to M_b would give an overall estimate of the nutritional status of the animal. Therefore, the objective of the present study was to determine if we could improve current intra-specific estimates of RMR in fasting king penguins by accounting for their structural size.

Materials and methods

Approval for all procedures was granted by the ethics committee of the French Polar Research Institute (IPEV) and of the Ministère de l'Environnement. The requirements of the UK (Scientific Procedures) Act 1986 were also followed. Our procedures also conformed to the Code of Ethics of Animal Experimentation in the Antarctic.

The experiments were carried out on Possession Island (Crozet Archipelago 46°25′ S, 51°45′ E) over the Austral summers 1998–1999, 2002–2003, and 2003–2004. Published and unpublished respirometry data on a total of 44 courting or breeding male ($N = 33$) and female ($N = 11$) king penguins, *A. patagonicus*, were used in this analysis (see Table 1).

Gender was determined by the song (Jouventin 1982) and later confirmed by genetic analysis (Avian Biotech International, Truro, Cornwall). Animals were caught within 1 day upon returning from a foraging trip (fed animals) or after a known fasting duration. All animals were caught either during the courtship period or while incubating. The stomach contents were removed about 12 h before respirometry experiments (Froget et al. 2001) for birds caught while incubating. Previous studies have shown that males have an empty stomach during the courtship period (Gauthier-Clerc et al. 2000), so it was assumed that all birds were post-absorptive. Most males ($N = 22$) were subjected to respirometry measurements at least twice and some as many as five times during fasting. RMR was measured while animals had been resting in a thermoneutral respirometer for at least 1 h until stable rates of oxygen consumption $(\dot{V}_{O_2})$ were obtained for at least 20 min. All tests were performed at the same time of the day and were completed before noon. In addition, we include RMR data measured without replication, in females ($N = 11$) and in males ($N = 11$) either before or during fasting from (Froget et al. 2001). The combined data set contained a total of 114 respirometry measurements after a fasting duration that ranged between 0 and 31 days in which M_b, measured immediately before a respirometry experiment, ranged between 9.2 and 16.2 kg.

When comparing morphometric measurements between individuals, species and studies, it is vital that measuring techniques are standardized. For this reason our aim was to standardize measurement techniques within and between people performing each measurement. The beak (L_{beak}, mm) was measured from the point of the jaw to the tip of the beak (Fig. 1a) and the foot (L_{foot}, mm) from the back to the end of the longest toe (Fig. 1b). The flipper ($L_{flipper}$, mm) was measured, fully extended, from the sternum to its tip (Fig. 1c). $L_{flipper}$ and L_{foot} were measured to the nearest 2 mm using a plastic measuring tape, while L_{beak} was measured to the nearest 1 mm using plastic vernier callipers. All measurements were taken on the left hand side of the animal while it was restrained in a prone position on a padded mat.

To evaluate the error in our measurement techniques within and between researchers we measured the same animals repeatedly. In a subset of 12 male birds, multiple measurements ($n = 3$) were performed on separate occasions by the same person. In a second subset, repeated measurements were taken in 18 male birds by 2 separate people ($n = 2$). This allowed us to estimate the repeatability (R), or intra-class coefficient (Sokal and Rohlf 1981), for each morphometric variable. The standard error of the mean (SEM) was estimated as outlined by Becker (1967). R provides an estimate of the measurement error within and between different people measuring the same trait. A significant and high R suggests that the experimental measurements are sufficiently repeatable for informative comparisons. In addition, a trait with a consistently higher R is a more reliable comparative measure.

Experimental protocol and respirometry

Details of the respirometry system have been given previously (Froget et al. 2001; Fahlman et al. 2004). Before the start of each experiment, the animal was placed in the respirometer which was behind a curtain to prevent disturbance of the animal due to movements by the observer. The bird was allowed to rest in the respirometer for at least 1 h until stable $\dot{V}_{O_2}$ and rates of CO_2 production $(\dot{V}_{CO_2})$ readings were obtained which were taken as the RMR. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated using standard equations (Depocas 1957; Withers 1977) as described in Froget et al. (2001). The average $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were estimated from changes in gas concentrations.

Data assessment and statistical analysis

All values are reported as means $\pm$ 1 standard deviation (SD), unless otherwise specified. Student's t test was

Table 1 Summary of body mass range (M_b, kg), number of animals (N), $\dot{V}_{O_2}$ (ml $O_2 \cdot$ min^{-1}) and source for all data used in the study. The numbers in parentheses are the number of experiments (n) on each group of animal

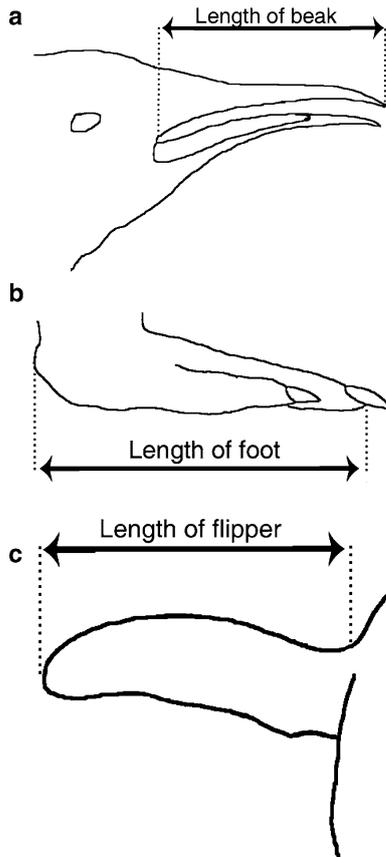| M_b	N (n)	Gender	$\dot{V}_{O_2}$	Source
13.4–10.2	11 (11)	Male	98.0 (11)	(Froget et al. 2001)
12.3–9.8	11 (11)	Female	82.3 (11)	(Froget et al. 2001)
14.9–9.7	5 (40)	Male	92.5 (40)	(Fahlman et al. 2004)
16.2–10.8	10 (24)	Male	99.7 (24)	(Fahlman et al. 2005)
13.6–9.2	7 (28)	Male	107.2 (28)	Unpublished

Fig. 1 Mensuration of **a** beak, **b** foot and **c** flipper in King Penguin

used to compare the difference between the means of two populations. Analysis of variance (ANOVA) with post-hoc Bonferroni multiple comparison testing was used when more than two populations were compared. Kolmogorov–Smirnov and F tests were used to check for the normality and equality of variance of the data. Departures from normality were corrected by appropriate transformations, e.g. log-transformation. In the case of unequal variances, Mann–Whitney or Kruskall–Wallis statistical tests were used. The R values were calculated using the method described in Krebs (1999) and the standard error of the mean (SEM) was estimated as described by Becker (1967).

To deal with multicollinearity between morphometric measurements we used principal component analysis (PCA, Jolliffe 2002). The resulting uncorrelated factors were used in a regression analysis to determine if predictions of resting $\dot{V}_{O_2}$ were improved. When body morphometrics are included in PCA, the first PC factor commonly has positive coefficients for all variables and is considered to be a measure of the structural size (Jolliffe 2002). The other factors tend to define certain aspects of the shape of the animal, presumably independent of the nutritional state.

We utilized mixed models regression, using a compound symmetry covariance structure to deal with correlation within animals (SAS, version 8, Littell et al. 1998). The method of maximum likelihood (ML) was used to search for the best parameters in which the log-likelihood (LL) is the value reported as a relative measure of the goodness of fit for each model. Multivariate mixed models use the likelihood ratio test to determine the significance of nested models. In the likelihood ratio test, significance is defined by increases in the LL values of the models (i.e. significantly smaller negative LL values). Finally, a goodness of fit test was used to assess the overall accuracy of the prediction equation.

The fiducial limit of statistical significance was set at the $P < 0.05$ level.

Results

Morphometrics

Figure 1 provides a schematic to show how we sampled L_{beak}, L_{foot} and L_{flipper}. We followed the directions of Stonehouse (1960) in the current study except when measuring the foot because we did not include the claw. The mean values for L_{beak}, L_{flipper} and L_{foot} are summarized in Table 2 and for each variable the mean value was greater for males than for females (t test, $P < 0.01$). None of the morphometric measurements showed any systematic changes with fasting duration ($P > 0.3$, repeated measures ANOVA).

R ($\pm$ SEM) estimated from 3 repeated measurements done by the same person in 12 animals were 0.89 ± 0.05 ($F_{11, 24} = 33.1$, $P < 0.01$) for L_{beak}, 0.60 ± 0.14 ($F_{11, 24} = 6.7$, $P < 0.01$) for L_{foot} and 0.74 ± 0.10 ($F_{11,24} = 12.7$, $P < 0.01$) for L_{flipper}. Using a t test with a Bonferroni correction for multiple comparisons, R was significantly higher for L_{beak} than L_{foot} ($P < 0.015$, $t > 3.0$). There was no difference in R for the other morphometric measures. When R was estimated after two repeated measurements performed by two different people on 18 animals it was 0.97 ± 0.02 ($F_{17, 18} = 60.5$, $P < 0.01$) for L_{beak}, 0.87 ± 0.06 ($F_{17, 18} = 14.9$, $P < 0.01$) for L_{foot}, and 0.95 ± 0.02 ($F_{17, 18} = 39.2$, $P < 0.01$) for L_{flipper}. Again, R for L_{beak} was significantly higher than L_{foot} ($P < 0.01$, $t > 2.4$) and again there was no difference in R across the other morphometric measures.

Principal component analysis

A PCA was performed on the morphometric variables. The information from the two first PCA factors

Table 2 Mean beak, flipper and foot (in mm) measurements from male ($n = 46$) and female ($n = 10$) king penguins

	Beak (mm)	Flipper (mm)	Foot (mm)
Males	124.8 ± 3.9	324.6 ± 11.0	174.9 ± 7.0
Females	119.6 ± 5.7	313.8 ± 9.3	168.7 ± 5.2
P	< 0.01	< 0.01	< 0.01

P values are t test, or Mann–Whitney in case of unequal variances, for differences between males and females

explained a total of 84% of the variation. The coefficients for the first factor were all positive while the second and third factor had different positive and negative signs for the coefficients.

Metabolic rate

The allometric exponent b of $\dot{V}_{O_2}$ was determined by calculating the linear regression of $\log(\dot{V}_{O_2})$ against $\log(M_b)$ (Schmidt-Nielsen 1997). There was a significant correlation between the $\log_{10}$-transformed metabolic rate $[\log(\dot{V}_{O_2})]$ and M_b $[\log(M_b)]$ (LL = -161.8, $P < 0.01$). The mean error of the residuals for these birds, the difference between the observed $\dot{V}_{O_2}$ from respirometry (mean 97.2 ml $O_2 \cdot min^{-1}$, Table 1) and the predicted value (114.2 ml $O_2 \cdot min^{-1}$) from the regression model ([observed − predicted]/observed), was -17.5%, with a range between -65.1 and 46.2%, and an absolute mean error of 24.4%. However, the partial residuals against M_b ($P < 0.01$) or number of days of fasting ($P < 0.5$) were not randomly distributed and there were departures from normality (Neter 1996). Gender was an important covariate when M_b was the only other covariate in the model ($P < 0.05$, LL = -159.8).

As an estimate of structural size and shape, the factors determined by PCA were used. Consequently, each PC factor was calculated as the sum of the product between each morphometric and its parameter. The two first factors were estimated as:

$$PC_1 = 0.49 \cdot L_{beak} + 0.59 \cdot L_{foot} + 0.64 \cdot L_{flipper} \qquad (1a)$$

and

$$PC_2 = 0.84 \cdot L_{beak} - 0.52 \cdot L_{foot} - 0.16 \cdot L_{flipper}, \qquad (1b)$$

where all morphometric measurements are in millimetre. A multivariate regression was used to determine if gender, or structural size and shape were significant covariates. A stepwise search suggested that a model including the first 2 factors was warranted ($P < 0.01$), while gender ($P > 0.3$) or the third PC factor were not important covariates ($P > 0.5$). Consequently, the best equation was (LL = -155.2):

$$\log(\dot{V}_{O_2}) = 1.74 + 1.39 \cdot \log(M_b) - 3.98 \cdot 10^{-3} \cdot PC_1 - 6.85 \cdot 10^{-3} \cdot PC_2, \qquad (2)$$

where PC_1 and PC_2 are the factors in Eq. 1a and 1b and estimated from L_{beak}, L_{foot} and $L_{flipper}$, all in millimetre. The mean error of the residuals for this equation was -4.0% (101.1 ml $O_2 \cdot min^{-1}$), with a range between -45.4–32.5%, and an absolute mean error of 14.4%. For Eq. 2, the partial residuals against M_b ($P > 0.2$) or number of days of fasting ($P > 0.2$) were randomly distributed and there were no departures from normality (Neter 1996).

Applying these equations to the data from a previous study (Fahlman et al. 2004), where 5 male birds were fasted between 24 and 31 days (Table 1, Fahlman et al. 2004), Eq. 2 in the current study underestimated RMR by 7.8%. Equation 1 from the previous study, used the heart rate (f_H) technique to estimate $\dot{V}_{O_2}$ and underestimated RMR by 4.0 % (Fahlman et al. 2004). The residuals from both of these equations were randomly distributed with fasting duration.

Discussion

Our objective was to improve allometric estimates of RMR in fasting king penguins using estimates of structural size and shape in addition to M_b. We used morphometric measures in a PCA yielding PCA factors that served as proxies for structural size and shape. Shape could be an important complement to structural size to reduce variability as shape may have important effects on RMR, e.g. a larger flipper may indicate a larger pectoral muscle which is likely to contribute to higher overall metabolic rate. Allometric equations were then generated between these morphometric indices and resting $\dot{V}_{O_2}$(ml $O_2 \cdot min^{-1}$), our estimate for RMR, before and during fasting to test whether the inclusion of morphological characteristics significantly improved prediction of RMR.

When the first and second PC factors were included in the multivariate regression together with M_b (Eq. 2), the estimate of RMR significantly improved. Thus, M_b and the first two PC factors had a significantly lower mean error of the residuals than using M_b alone (i.e. 17.5 vs. 4.0%). Using Eq. 2, the partial residuals against M_b or number of days of fasting were randomly distributed, showing that the error of the estimate did not change throughout the fasting period. Thus, the use of factors independent of the nutritional state of the bird (structural index) together with M_b (structural and nutritional) allows removal of the effect of fasting duration (nutritional status) from the relationship between $\dot{V}_{O_2}$ and M_b. Inclusion of gender, on the other hand, was not warranted as it did not improve the fit to Eq. 2. Consequently, differences in RMR between males and females are due to differences in size and shape.

This method relies on the ability to accurately measure M_b, $L_{flipper}$, L_{beak} and L_{foot} and assumes that $L_{flipper}$ L_{beak} and L_{foot} do not change in adult birds during fasting or while foraging. Our application of R suggests that $L_{flipper}$, L_{beak} and L_{foot} are highly repeatable measurements both when taken by a single person and by different people. Therefore, all these measurements are reliable indicators of body size and shape of king penguins. In addition, repeated measurements on the same bird throughout the fasting period showed that these morphometrics did not change systematically with fasting. The morphometric measures $L_{flipper}$, L_{beak} and L_{foot} were significantly larger in males than in females in the present study (Table 2). This agrees with previous work showing sexual dimorphism in L_{beak} in king penguins from South Georgia (male 136.9 ± 5.8 mm, $n = 67$;

female 128.2 ± 5.3 mm, $n = 55$; Stonehouse 1960). However, measurements of L_{beak}, L_{foot} (male 186.3 ± 7.3 mm; female 177.8 ± 6.8 mm) and $L_{flipper}$ (male 340.6 ± 8.9 mm; female 330.2 ± 9.8 mm) measured by Stonehouse (1960) are all significantly larger than those in the current study ($P < 0.05$, t test). In contrast to Stonehouse (1960), we decided not to include the claw as a structural component of L_{foot} since it can change in size and/or be lost and replaced. The claw extends approximately 0.5–1 cm from the end of the toe and this could therefore explain the differences in L_{foot} observed in the current study and those measured by Stonehouse (1960). However, it is concluded that the differences in measurements of $L_{flippper}$ and L_{foot} indicate differences in morphology between the populations on South Georgia and on Possession Island. This highlights the power of the method employed in the present study and suggests that these two geographically distinct populations of king penguins differ in RMR. That is, a 14 kg male bird in Crozet, using the average morphometrics from the present study, would have a RMR of 130 ml $O_2 \cdot min^{-1}$ while a 14 kg male bird in South Georgia would have a RMR of 103 ml $O_2 \cdot min^{-1}$ (100 ml $O_2 \cdot min^{-1}$ reducing L_{foot} by 1 cm). Admittedly, this assumes that Eq. 2 applies to all populations of king penguins but potentially this method represents a useful tool for evolutionary biologists and ecologists.

In a previous study, an equation was created that predicted $\dot{V}_{O_2}$ in penguins while fasting on land using the f_H technique (Eq. 1, Fahlman et al. 2004). In comparing Eq. 1 from Fahlman et al. (2004) with Eq. 2 from the current study, we used data from Fahlman et al. (2004) which consisted of 5 animals each measured 5 times throughout a 24–31 day fasting period. Equation 1 from Fahlman et al. (2004) underestimated RMR by 4.0% (Eq. 1, Fahlman et al. 2004) while Eq. 2 from the current study underestimated RMR by 7.8%. Thus, the accuracy of this new method to estimate RMR, using only M_b and three morphometric measurements compares well with the f_H-technique. While this new method cannot estimate $\dot{V}_{O_2}$ in active animals, the implications of this simple concept are important as they reduce the handling time, workload and cost when measuring RMR in the field. For studies that would like to estimate RMR throughout the fasting period, M_b needs to be measured repeatedly. As M_b can be measured with minimal disturbance to the bird (Gendner et al. 1992; Handrich et al. 1995), this method has the advantage of reducing handling. Alternatively, if the fasting duration is known, M_b can be predicted using the initial M_b and the mass-specific daily decrease of M_b which during the first 5 days (phase I) of fasting is 22.9 g kg^{-1} day^{-1} and then levels off to a steady rate of 11.6 g kg^{-1} day^{-1} (Fahlman et al. 2004). In addition, as we showed previously, RMR in fed penguins is the same in air and water (Fahlman et al. 2005) so this method can be used to estimate RMR in fed birds during a foraging trip. Whether adjusting M_b for structural size allows the estimation of RMR in other species that express a sig-

nificant variation in M_b and structural size remains to be determined.

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