Effect of fasting on the $\dot{V}_{O_2}$-f_h relationship in king penguins, *Aptenodytes patagonicus*

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Fahlman, A., Y. Handrich, A. J. Woakes, C.-A. Bost, R. Holder, C. Duchamp, and P. J. Butler. Effect of fasting on the $\dot{V}_{O_2}$-f_h relationship in king penguins, *Aptenodytes patagonicus*. *Am J Physiol Regul Integr Comp Physiol* 287: R870–R877, 2004. First published June 3, 2003; 10.1152/ajpregu.00651.2003.—King penguins (*Aptenodytes patagonicus*) may fast for up to 30 days during their breeding period. As such extended fasting may affect the relationship between the rate of O_2 consumption ($\dot{V}_{O_2}$) and heart rate (f_H), five male king penguins were exercised at various speeds on repeated occasions during a fasting period of 24–31 days. In addition, $\dot{V}_{O_2}$ and f_H were measured in the same animals during rest in cold air and water (4°C). $\dot{V}_{O_2}$ and f_H at rest and $\dot{V}_{O_2}$ during exercise decreased with fasting. There was a significant relation between $\dot{V}_{O_2}$ and f_H ($r^2 = 0.56$) that was improved by including speed, body mass (M_b), number of days fasting (t), and a cross term between f_H and t ($r^2 = 0.92$). It was concluded that there was a significant change in the $\dot{V}_{O_2}$-f_H relationship with fasting during exercise. As t is measurable in the field and was shown to be significant and, therefore, a practical covariate, a regression equation for use when birds are ashore was obtained by removing speed and M_b. When this equation was used, predicted $\dot{V}_{O_2}$ was in good agreement with the observed data, with an overall error of 3.0%. There was no change in the $\dot{V}_{O_2}$-f_H relationship in penguins at rest in water.

sea bird; exercise; heart rate technique; metabolic rate; body composition

THE USE OF HEART RATE (f_h) to estimate metabolic rate in the field has recently received much attention (15, 18, 22, 29) and is based on the relationship between rate of O_2 consumption ($\dot{V}_{O_2}$) and f_H as formulated in the Fick equation for convection of O_2 in the cardiovascular system (5). Unfortunately, it cannot always be assumed that the relationship between f_H and $\dot{V}_{O_2}$ for a species remains the same under all conditions. The relationship may be affected by several factors, such as gender (15), type and level of activity (7, 29), physiological state (fasting, breeding, and molting) (15, 18), and seasonal changes (23).

In king penguins (*Aptenodytes patagonicus)*, the relationship was significantly different between males returning from a foraging trip and males that had been on the nest with a chick for 14–17 days (15). It was postulated that this difference was the result of fasting when the animal was ashore and probably resulted from a change in body composition. Thus the aim of the present study was to test the hypothesis that the relationship between f_H and $\dot{V}_{O_2}$ changes during fasting in king penguins. This was achieved by determining the $\dot{V}_{O_2}$-f_H relationship at various levels of exercise on a treadmill at repeated intervals in five male king penguins during an extended fasting period. As the animals alternate periods ashore with periods at sea during the breeding period, resting $\dot{V}_{O_2}$ and f_H were also determined in air and water for the same animals in a separate experiment.

MATERIALS AND METHODS

Animals

Ethical approval for all procedures was granted by the Ethics Committee of the French Polar Research Institute and the Ministère de l'Environnement. We also carefully followed the requirements of the United Kingdom (Scientific Procedures) Act 1986, especially those set out by the Home Office in the Official Guidance on the Operation of the Act. As our benchmark, we followed guidance to researchers using similar methods in the United Kingdom.

The experiments were carried out on Possession Island (Crozet Archipelago 46°25′ S, 51°45′ E) over the austral summer 2002–2003. A total of five courting male king penguins were used in the experiments. Gender was determined by the song (24) and later confirmed by genetic analysis (Avian Biotech International, Truro, Cornwall, UK). All birds were caught directly on the beach, outside the breeding site, in early February at the beginning of courtship. Thus these animals were late breeders. At this stage in the courtship, mate choice had not been made, and the female immediately commenced courtship with other partners. The birds were caught in the afternoon on the day before the first experiment and were weighed. We used only birds with an initial mass >13.0 kg, a body mass known to allow male king penguins to fast for ≥1 mo while incubating the egg (17). The lengths of the flipper ($L_{flipper}$), bill (L_{bill}), and foot (L_{foot}) were measured according to standard techniques (31). Repeated measurements of $L_{flipper}$, L_{bill}, and L_{foot} were made to reduce measurement errors, and all measurements were made on the left side of the animal. A 0.3-ml blood sample from the brachial flipper vein was taken for gender determination. Each bird was fitted with a temporary plastic flipper band for recognition and placed in a wooden enclosure (3 × 3 m), where it was kept for the duration of the fasting period.

All experimental birds repeated a treadmill test five times (treadmill experiments) over a period of 24–31 days (including the 1st day of capture; Tables 1 and 2). Throughout the fasting period, the animals had no access to water other than from rainfall. Each bird had ≥3 and ≤9 days of rest before repeating the treadmill test, and the numbers of days of fasting before each test are summarized in Table 2. In addition, $\dot{V}_{O_2}$ and f_H of each bird were measured in air and water (air/water experiment) during rest at 4°C twice throughout the fasting period (*tests A* and *B*). Body mass (M_b) was determined for each animal before each experiment (Table 2). The M_b values of the birds during *tests A* and *B* were 12.1 ± 0.6 (mean ± SD) and 10.4 ± 0.5

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Table 1. *Morphometry of king penguins used to determine $\dot{V}_{O_2}$-f_H relationship during an extended period of fasting*

Bird No.	Initial M_b, kg	Final M_b, kg	Fast Duration, days	L_{bill}, mm	L_{foot}, cm	$L_{flipper}$, cm	cM_b, kg
8	14.5	9.8	31	122.8±0.9 (6)	17.4±0.5 (6)	33.0±0.5 (6)	9.09
9	14.2	9.4	26	125.7±1.1 (5)	17.5±0.5 (6)	33.0±0.2 (5)	9.40
10	14.9	10.3	31	126.4±0.3 (5)	17.1±0.6 (7)	33.4±0.3 (6)	9.46
11	14.1	10.0	26	118.0±0.5 (3)	16.7±0.3 (3)	32.9±0.2 (3)	8.61
12	13.3	9.7	24	125.6±0.6 (4)	17.4±0.2 (4)	33.3±0.3 (4)	9.38
Mean ± SD	14.2±0.6	9.8±0.3	27.6±3.2	123.7±3.5	17.2±0.3	33.1±0.2	9.19±0.35

Morphometric values are means ± SD, and the number of repeated measurements are in parentheses. M_b, body mass; cM_b, critical M_b (17); L_{bill}, $L_{flipper}$, and L_{foot}, length of bill, flipper, and foot, respectively.

kg, respectively. The animals had fasted for 5–12 (10.0 ± 3.9) and 11–25 (22.6 ± 1.3) days before *tests A* and *B*, respectively.

M_b of each bird was continuously monitored to detect any increase in the mass specific daily change in M_b ($dM_b/M_b \cdot dt$), a sign that the animal is entering a metabolic state of high protein breakdown, the so-called phase III of fasting, which is associated with a signal to refeed and abandon the egg (19, 27). At the transition between phases II and III, M_b decreases below a critical M_b (20). In the present study, the mean M_b on release was 7% above the critical M_b (Table 1) as defined by Gauthier-Clerc et al. (17), and no increase of $dM_b/M_b \cdot dt$ was detected in any bird just before its release into the wild after completion of the experiment. Thus the birds were in phase I or phase II of fasting during the experiments.

Experimental Protocol

At the start of each test, the animal was fitted with an externally mounted heart rate data logger (HRDL) (33) placed in a protective plastic casing (30 × 36 × 12 mm, 28 g), as described by Froget et al. (15). The HRDL calculated and stored the average f_H once per second.

After the HRDL was attached, the animal was placed in the respirometer, which was placed behind a curtain to prevent disturbance of the animal due to movement of the observer. A hole in the curtain allowed continuous observation of the penguin. The bird was allowed to rest in the respirometer for ≥1 h until stable $\dot{V}_{O_2}$ and CO_2 production ($\dot{V}_{CO_2}$) readings were obtained over ≥20 min. Next, the penguin was walked on the treadmill, which was the floor of the respirometer, at 0.3, 0.7, 1.0, 1.5, and 1.8 km/h. The sequence of walking speeds was assigned at random between birds, but the sequence of speeds was the same within birds between tests. There was no attempt to walk the animals at their maximum sustainable speed. The animal walked at each speed until steady values for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were obtained for ≥5 min, usually within 15–20 min after the animal started to walk. Each walking session at a given speed was separated by a period of rest, with the bird left undisturbed until $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ had again reached resting values.

Table 2. *Total number of days fasting for each male king penguin used to determine $\dot{V}_{O_2}$-f_H relationship during the fasting period (tests) and mean M_b for each test*

Bird No.	Days Fasting				
	Test 1	*Test 2*	*Test 3*	*Test 4*	*Test 5*
8	1	6	14	22	31
9	1	5	13	20	26
10	1	8	15	22	31
11	1	7	13	20	26
12	1	4	12	19	24
Mean ± SD	1.0±0.0	6.0±1.6	13.4±1.1	20.6±1.3	27.6±3.2
M_b	13.8±0.6	12.6±0.5	11.5±0.5	10.7±0.5	9.8±0.3

M_b values are means ± SD.

All tests were performed at the same time of the day and were completed before noon to avoid possible diurnal effects and to reduce the effect of the outside temperature on the temperature inside the respirometer.

Respirometry in the Treadmill Experiments

$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ when the birds were on the treadmill were measured using a flow-through respirometer system similar to that used by Froget et al. (15) but with a few modifications.

The internal dimensions of the respirometer, including frame and Plexiglas box, were 80 × 46 × 86 cm (= 316 liters, length × width × height). The excurrent flow rate was ∼80 l/min, and a subsample of this gas passed via a canister of anhydrous $CaSO_4$ (W. A. Hammond Drierite, Xenia, OH) to a paramagnetic O_2 and an infrared CO_2 analyzer (Servomex 1440). The gas analyzers were calibrated before and after each experiment using pure N_2, ambient air (20.9% O_2), and 1% CO_2 in N_2 made up by a gas-mixing pump (model 2M301/a-F, Wösthoff, Bochum, Germany). The temperature and humidity inside and outside the box and the ambient pressure were measured using suitable sensors (Farnell Electronics). The ranges of these variables were 10.1–19.8°C, 31.9–100%, and 98.7–101.4 kPa, respectively. A solenoid valve (RS Component) switched between sampling excurrent and ambient gas to allow compensation due to any drift in the gas analyzers. The accuracy of the system was determined by N_2-dilution tests (14), where N_2 and CO_2 were used as the dilution gas. These tests showed that the difference between the observed and expected values was within 2% and confirmed that the system was properly sealed. The time constant of the system was ∼4 min, including the volume of the respirometer and the polyvinylchloride tubing to the analyzers. The time required to reach a 95% fractional transformation to a new steady state was computed to be 3.2 times the time constant, or ∼13 min.

During an experiment, the output signals of the gas analyzers and flowmeters and the humidity, temperature, and pressure sensors were passed to a purpose-built signal conditioner. The processed signals were passed to a computer via an analog-to-digital converter (DAQ500 card, National Instruments). Data were sampled, displayed, and analyzed every 2 s using Lab VIEW 5.0 software (National Instruments, Austin, TX). All flows were corrected to standard temperature (273 K) and pressure (101.3 kPa) dry (STPD), and the data were saved to a file.

Respirometry in the Air/Water Experiments

Resting values of $\dot{V}_{O_2}$ with the birds in air or water were determined as described by Barré and Roussel (2) and Froget et al. (16). Briefly, the animal was placed in a plastic bucket in a thermostatic chamber and fitted with an opaque Plexiglas hood over the head. The hood attached to the lid, which covered the bucket, and the lid attached securely to the bucket. During all experiments, the bird rested while standing in the container in air or submerged in water. During submersion, water covered the animal from the feet to the neck.

A vacuum pump (Miniseco SV/SD 1000, Busch, Switzerland) was attached to the hood via a long section of polyvinylchloride tubing (~5 m), which created a flow of air through the hood of ~45–50 l/min. The flow was measured using a variable flowmeter (model S7530, Houdec) and corrected to STPD. A second vacuum pump (Miniport, KNF Neuberger, Freiburg, Germany) removed a subsample of the excurrent gas, which, once passed through a canister of drying agent (Drierite), was analyzed for O_2 (model 1100, Servomex) and CO_2 (model 1410B, Servomex) concentrations. The gas analyzers were calibrated before and after each experiment using ambient air (20.9% O_2), pure N_2, and 0.6% CO_2 in N_2 (Air Liquide, Alphagaz, Paris, France). Temperature and humidity of the excurrent gas were measured by a custom-built thermistor and hygrometer, respectively. A manual valve allowed switching between sampling excurrent and ambient gas to compensate for any drift in the gas analyzers.

During an experiment, data were sampled, displayed, and analyzed every 10 s on a computer using Labtech Notebook software. The birds were initially exposed to room air for 60 min and then submerged in water. For *tests A and B,* air temperatures were 4.6 ± 0.3°C and 3.7 ± 0.2°C, respectively. Stable values for $\dot{V}O_2$ and $\dot{V}CO_2$ in air were usually observed after ~20 min. The birds were then submerged in water at a mean temperature of 4.9 ± 0.6°C and 4.4 ± 0.5°C for *tests A* and *B,* respectively. Animals were submerged until steady values for $\dot{V}O_2$ and $\dot{V}CO_2$ were obtained for ≥20 min, and stable values were usually observed after 20–40 min. Body temperature (°C) was measured using a thermistor (accuracy ±0.2°C) that was placed ~50 cm into the esophagus, and the connecting lead was fixed with Tesa tape at the opening of the bill. Body temperatures in air and water were 37.7 ± 0.5°C and 36.9 ± 1.3°C, respectively. The animals were randomly assigned to start testing in the morning (0300–0600) or afternoon (1200–1400). All animals were held captive and were fasting between tests.

Data Assessment and Statistical Analysis

Values are means ± SD, unless otherwise stated. Student's *t*-test was used to compare the difference between the means of two populations. Analysis of variance (ANOVA) with Bonferroni's multiple comparison adjustments was used when more than two populations were compared. Kolmogorov-Smirnov and *F*-tests were used to check for normality and equality of variance of the data. Departures from normality were corrected by appropriate transformations, e.g., logarithmic transformation. In the case of unequal variances, Mann-Whitney or Kruskall-Wallis statistical tests were used.

$\dot{V}O_2$ and $\dot{V}CO_2$ were calculated using standard equations (15). The recorded values for f_H were averaged over the same interval as the values used to calculate $\dot{V}O_2$. Temporal changes in the $\dot{V}O_2$-f_H relationship were analyzed using the average values for f_H and $\dot{V}O_2$, together with various covariates for each animal and experiment, in a multivariate regression analysis.

Multivariate least squares regression models were used to determine the effect of fasting on the $\dot{V}O_2$-f_H relationship, with $\dot{V}O_2$ as the dependent variable and five experimental variables [f_H, respirometer temperature, speed (U), M_b, and t] as independent fixed covariates. Initially, a univariate analysis on each independent variable was performed, and only those variables with $P < 0.20$ (Wald's tests) were considered in the multivariate analysis. Stepwise techniques were used to search for the best model. Nested regressions were compared with each other by *F*-tests, with significant difference assigned at $P < 0.05$. For the forward step, each variable was analyzed sequentially, starting from only the *y*-intercept term and adding each successive variable in order of greatest statistical significance. This was followed by an *F*-test to determine whether there had been a significant improvement. The backward step began with all variables in the model, and any that were not significant were eliminated. This tested the robustness of the model by confirming that the best-fit model from the backward and forward step included the

same variables and had the same parameter estimates for those variables. Cross terms were considered to determine possible interaction effects in the relation between $\dot{V}O_2$ and the covariates. The parameters from the most parsimonious model were considered to draw conclusions about the effect of fasting in a laboratory setting, while modified models were considered as alternatives in the field. The models were analyzed and corrected for departures from normality, outliers, and linearity as detailed by Neter et al. (28).

Statistical analyses were performed using NCSS 2000 (NCSS, Kaysville, UT), Minitab (version 13.32, Minitab, State College, PA), or SAS (version 8, SAS Institute, Cary, NC) statistical software. Acceptance of significance was set at $P < 0.05$, and $0.05 < P < 0.1$ was considered a trend (9). A statement that mean values were different signifies that they were significantly different.

RESULTS

Animals and Morphology

Morphological summary statistics are presented in Table 1. In Table 2, M_b and days of fasting are shown for the animals when used in the treadmill experiments.

M_b During Fasting

M_b losses throughout the fasting period for the five king penguins are summarized in Fig. 1. M_b decreased linearly beyond *day 5* of the fasting period (Fig. 1*A*), and there was a significant correlation between log(M_b) and *t* ($r^2 = 0.80$; Fig. 1*B*). Mass-specific daily change of M_b ($n = 70$) was biphasic and decreased rapidly during the first 5 days (phase I of fasting) from an average of 22.9 ± 9.4 g·kg^{-1}·day^{-1} ($n = 20$) and then leveled of to a more-or-less constant rate of 11.6 ± 2.9 g·kg^{-1}·day^{-1} ($n = 50$; Fig. 1*C*) throughout the remaining fasting period (phase II of fasting). No bird showed an increase in dM_b/M_b·d*t* before its release, indicating that no bird entered phase III of fasting.

Treadmill Experiments

Rest. After a period of exercise, neither resting $\dot{V}O_2$ nor resting f_H was correlated with the preceding speed or sequence of the resting periods ($P > 0.9$ by 1-way ANOVA for $\dot{V}O_2$ and $P > 0.4$ for f_H). This suggests that the resting period was sufficiently long to allow $\dot{V}O_2$ to return to resting values and that there was no carryover effect. As there were no systematic changes, the repeated measurements of resting $\dot{V}O_2$, f_H, and respiratory exchange ratio ($\dot{V}O_2$/$\dot{V}CO_2$) were averaged for a given bird, and the mean value of the five repeated resting periods was used as the representative resting value for that test (Table 3).

During resting periods, the animals were mostly standing or occasionally laying prone. While standing, the animals were mostly resting in a hunched posture, similar to that observed in the field, or at times preening or investigating the respirometer. For all animals, the minimum values for $\dot{V}O_2$ and f_H were observed during these resting periods. Resting $\dot{V}O_2$ decreased 11–17% (Table 3) between tests, while M_b decreased 7–9% (Table 1), and the overall changes were 48% and 29%, respectively, from *test 1* to *test 5*. Between *test 2* and *test 5*, the decrease was 39% for $\dot{V}O_2$ (Table 3) and 22% for M_b (Table 1). For f_H, on the other hand, there was no systematic change between tests, but during *test 5*, f_H was significantly lower than that during *test 1* or *test 3* by 31% and that during *test 2* by 27% (Table 3; $P < 0.05$ in each case).
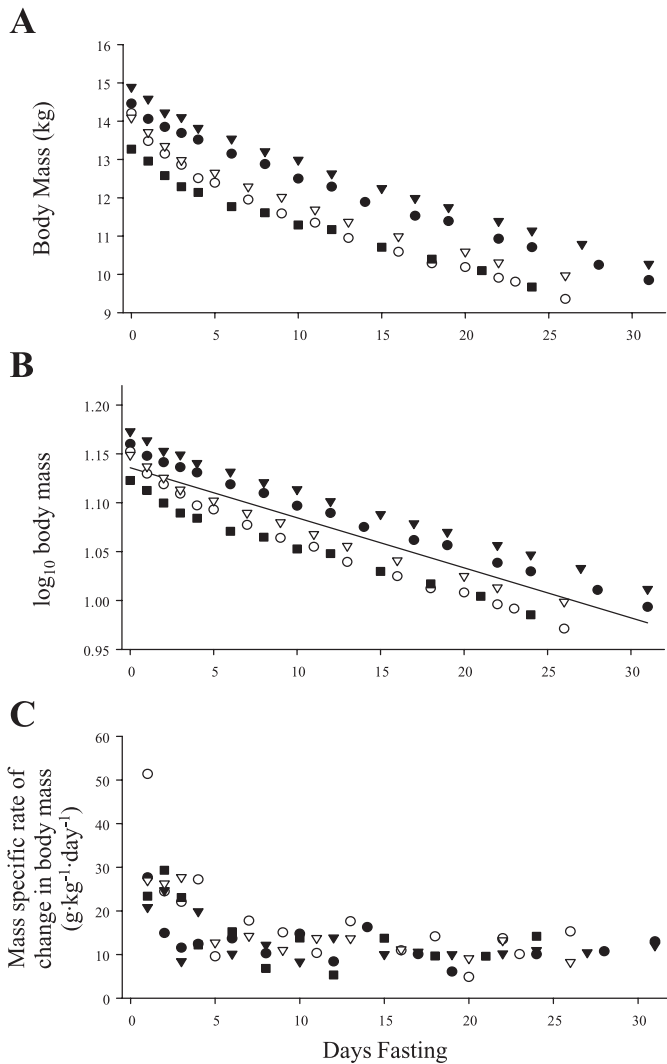
A

B

C

Fig. 1. Linear (*A*) and $\log_{10}$-transformed (*B*) daily body mass and mass-specific daily change in body mass (*C*) of 5 male king penguins used to determine O_2 consumption ($\dot{V}_{O_2}$)-heart rate (f_H) relationship during an extended period of fasting. Different symbols represent different birds.

Changes in f_H and $\dot{V}_{O_2}$ with speed. Except for *test 1*, the maximum values in the present study for $\dot{V}_{O_2}$ and f_H were attained at the highest or second-highest speed (Fig. 2*A*). When the data for all tests and animals were pooled (rest included),

$\dot{V}_{O_2}$ changed linearly with speed ($\dot{V}_{O_2} = 130.5 + 104.7 \cdot U$, $r^2 = 0.47$, $P < 0.01$, $n = 150$; Fig. 2*A*). For f_H, the linear regression when the resting values were omitted was as follows: $f_H = 149.7 + 32.5 \cdot U$ ($r^2 = 0.14$, $P < 0.01$; Fig. 2*B*), and the slopes changed between tests ($P < 0.01$, $F_{4,115} = 4.38$). However, when the data for *test 1* were omitted, there was no difference between the slopes ($P > 0.5$, $F_{3,92} = 0.06$) or the intercepts ($P > 0.3$, $F_{3,95} = 1.20$).

The coefficient of variation (SD/mean) of f_H for resting birds and each speed increased with test number ($\dot{V}_{O_2} = 77.3$, $P < 0.01$ by Kruskall-Wallis 1-way ANOVA on ranks).

$\dot{V}_{O_2}$-f_H relationship during rest and exercise. There was no change in the O_2 pulse (OP, ml O_2/heartbeat) with fasting at rest ($P > 0.5$, $F_{4,20} = 0.77$); thus the resting $\dot{V}_{O_2}$-f_H relationship did not change with fasting. With the exception of one test for one animal ($r^2 = 0.56$, $P = 0.08$), good correlations were observed between f_H and $\dot{V}_{O_2}$ for each animal and each test ($r^2 = 0.70$–0.98). Between animals, there was no difference between the slopes within any one test ($P > 0.5$ ANCOVA), but there was a significant difference in the intercepts ($P < 0.01$). For this reason, we used a random-effects model, with the intercepts treated as a random sample from a normal distribution. The appropriate ANCOVA would therefore allow the intercept to be entered as a random effect, and an additional random term was introduced into the model (18). When all animals and all tests were combined, there was a significant correlation between f_H and $\dot{V}_{O_2}$ ($P < 0.01$, $r^2 = 0.38$, $n = 150$). However, the residuals from this regression were not normally distributed, and $\dot{V}_{O_2}$ and f_H were $\log_{10}$ transformed, which improved the fit ($r^2 = 0.56$) and normalized the residuals. There was a significant difference in the slopes between tests for the combined data ($P < 0.01$, $F_{4,144} = 52.9$; Fig. 3), suggesting that the relationship changed between tests. When *test 1* was removed, there were no differences in the slopes ($P > 0.1$, $F_{3,115} = 1.11$; Fig. 3) between tests.

Univariate analysis showed that t ($r^2 = 0.26$, $P < 0.01$), $\log(M_b)$ ($r^2 = 0.27$), and a nonlinear transformation of U ($U' = 1 - e^{-U}$; $r^2 = 0.63$, $P < 0.01$) were important covariates. Temperature in the respirometer was not an important covariate ($P > 0.7$, $r^2 = 0.02$) and not further considered.

Stepwise regression was used to finally include $\log(f_H)$, t, U, $\log(M_b)$, and an interaction term between $\log(f_H)$ and t ($r^2 = 0.90$). Including U' instead of U led to an improvement of the

Table 3. *Resting $\dot{V}_{O_2}$, RER, f_H, maximum $\dot{V}_{O_2}$ and f_H and percent increase between resting and maximum $\dot{V}_{O_2}$ used to determine the $\dot{V}_{O_2}$-f_H relationship during the fasting period*

Test No.	Resting $\dot{V}_{O_2}$, ml/min	RER	Resting f_H, beats/min	Maximum $\dot{V}_{O_2}$, ml/min	Maximum f_H, beats/min	Δf_H, %	$\Delta \dot{V}_{O_2}$, %
1	127.6±26.6	0.76±0.12	92.9±13.7	418.8±66.1	212.1±29.4	132±48	236±62
2	107.2±9.2*	0.76±0.05	87.6±15.6	359.7±27.2	214.0±40.7	149±55	238±44
3	95.3±12.8*	0.74±0.04	92.9±24.4	295.1±22.9*	226.2±58.9	146±41	212±25
4	79.1±3.7*†	0.75±0.03	82.5±36.8	289.3±40.3*	233.7±30.6	211±84	267±58
5	65.8±5.2*†‡	0.69±0.03	63.8±28.2*†‡	232.1±31.1*†‡	215.0±30.2	272±116*†‡	255±57
P	<0.01	>0.9	<0.05	<0.01	>0.6	<0.01	>0.4

Values are means ± SD of 5 king penguins. RER, respiratory exchange ratio ($\dot{V}_{O_2}$/$\dot{V}_{CO_2}$). *P* values were determined by repeated-measures ANOVA, with test number as within-group fixed factor. *Significantly different from *test 1;* †significantly different from *test 2;* ‡significantly different from *test 3* ($P < 0.05$, Bonferroni's multiple comparison).
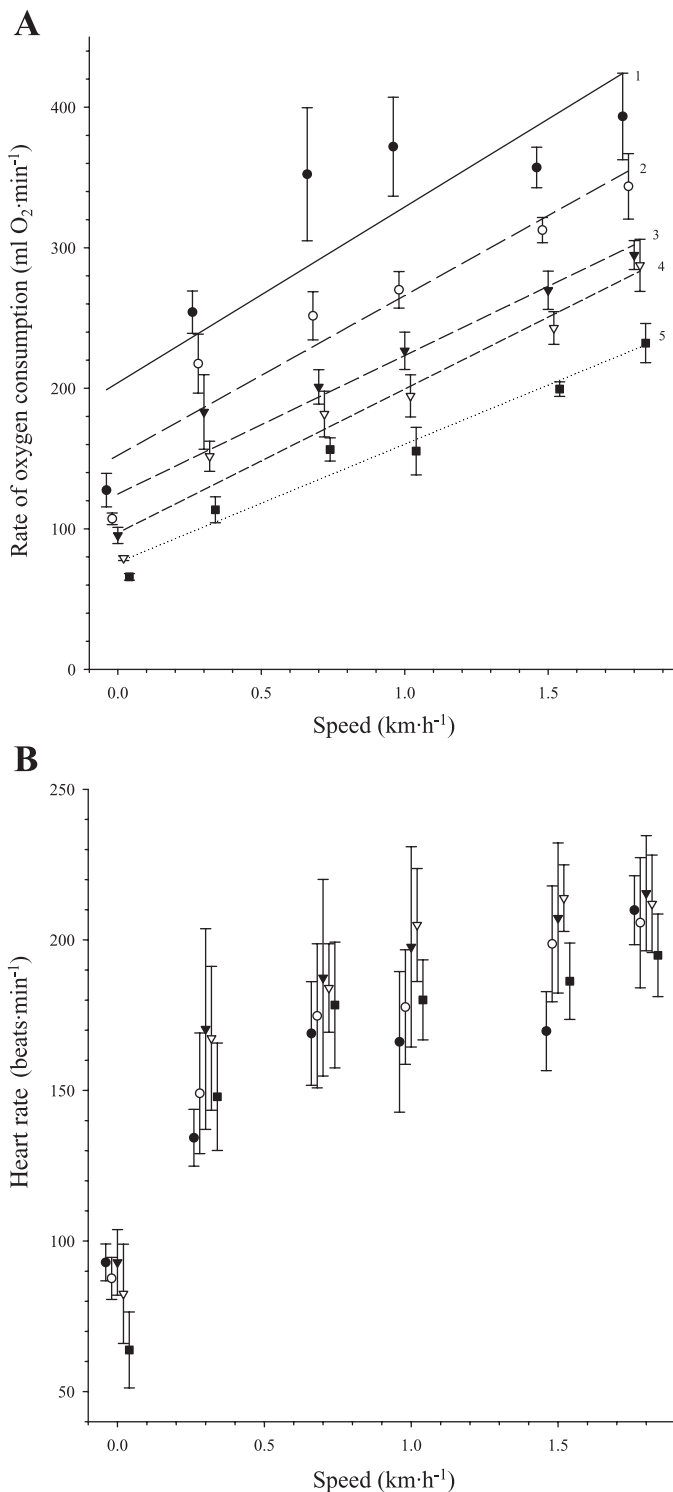
A

B

Fig. 2. Rate of oxygen consumption (*A*) and heart rate (*B*) vs. treadmill speed for 5 repeated treadmill experiments (*tests 1–5*) for 5 male king penguins during 24–31 days of fasting. ●, *Test 1*; ○, *test 2*; ▼, *test 3*; □, *test 4*; and ■, *test 5*. Regressions were calculated from raw data, but symbols represent means ± SE for each test. Values for speed at each test are slightly offset to ease presentation, but speeds were 0, 0.3, 0.7, 1.0, 1.5, and 1.8 km/h. Numbers in *A* represent test number for each regression line.
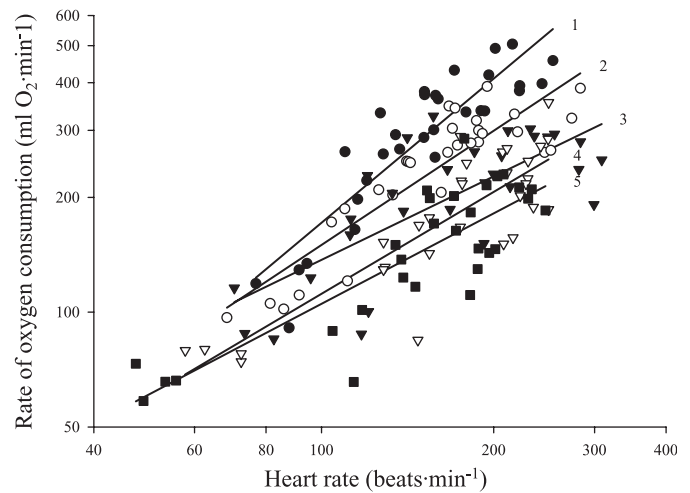

Fig. 3. Rate of oxygen consumption vs. heart rate for 5 repeated treadmill experiments (*tests 1–5*) for 5 male king penguins during 24–31 days of fasting. Data are plotted on a double logarithmic scale to ease interpretation. See Fig. 2 legend for explanation of symbols. Numbers represent test number for each regression line. Regression equations are as follows: $\log(\dot{V}_{O_2}) = -0.49 + 1.35 \cdot \log(f_H)$ ($r^2 = 0.86$) for *test 1*; $\log(\dot{V}_{O_2}) = -0.33 + 1.23 \cdot \log(f_H)$ ($r^2 = 0.93$) for *test 2*; $\log(\dot{V}_{O_2}) = -0.37 + 1.21 \cdot \log(f_H)$ ($r^2 = 0.89$) for *test 3*; $\log(\dot{V}_{O_2}) = 0.03 + 1.00 \cdot \log(f_H)$ ($r^2 = 0.82$) for *test 4*; $\log(\dot{V}_{O_2}) = 0.27 + 0.87 \cdot \log(f_H)$ ($r^2 = 0.80$) for *test 5*.

fit ($r^2 = 0.92$), but neither U nor U' is usable in the field. Thus, for use in the field, the equation was modified by removing M_b, U, or U' and including polynomials and interaction terms. Polynomials and interaction terms were added until there was no additional improvement in the fit as determined by an *F*-test (28). The best model was as follows

$$\log(\dot{V}_{O_2}) = -0.279 + 1.24 \cdot \log(f_H) + 0.0237 \cdot t \\ - 0.0157 \cdot \log(f_H) \cdot t \ (r^2 = 0.81) \quad (1)$$

The percent errors of the residuals, the relative difference between the observed and predicted values {[(observed − predicted)/observed]·100}, from *Eq. 1* was 3.0%, with a range of −95.9% to 42.1% and an absolute mean error of 19.3%. The partial residuals were randomly distributed against all covariates, and there were no departures from normality (28).

Resting in Air and Water Experiments

$\dot{V}_{O_2}$ and f_H during rest in air/water. Resting values of $\dot{V}_{O_2}$ for the birds when in air in the air/water experiments (Fig. 4) were similar to those during the treadmill experiments ($P > 0.6$ by 2-tailed *t*-test) for tests where the birds had a similar M_b and t, i.e., *test A* (98.0 ± 16.4 ml O_2/min, 10.0 days, $n = 5$) compared with *tests 2* and *3* (Tables 1 and 2, $n = 10$) and *test B* (72.8 ± 10.9 ml O_2/min, 22.6 days) compared with *tests 4* and *5* (Tables 1 and 2). The ranges of mean resting f_H values in air for *tests A* and *B* were 64.1–110.2 and 50.6–93.0 beats/min, respectively (Fig. 4, *A* and *B*). In water, the mean resting $\dot{V}_{O_2}$ and f_H were 149.2–191.1 ml O_2/min and 97.3–135.3 beats/min for *test A* and 111.6–243.0 ml O_2/min and 68.3–144.1 beats/min for *test B* (Fig. 4, *A* and *B*).

Differences between resting values in air and in water. To evaluate differences in $\dot{V}_{O_2}$, f_H, and OP for animals resting in air or water, the resting values for these variables were analyzed using a repeated-measures ANOVA (SAS version 8),
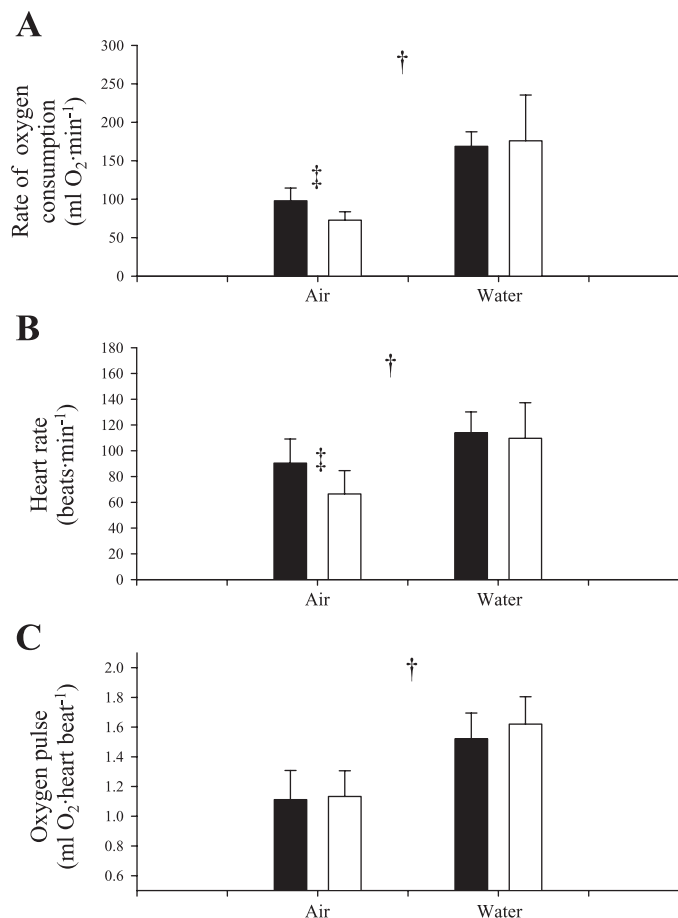
A

B

C

Fig. 4. Rate of oxygen consumption (*A*), heart rate (*B*), and oxygen pulse (*C*) for animals resting in air or in water at 4°C for *test A* (solid bars) and *test B* (open bars). Values are means ± SD. †Significant difference between air and water; ‡significant difference between tests ($P < 0.05$, repeated-measures ANOVA followed by Bonferroni's multiple comparison).

with test number and M_b as fixed effects and animal as a random effect. $\dot{V}_{O_2}$ and f_H increased between air and water ($P < 0.01$, $F_{1,13} = 38.31$ for $\dot{V}_{O_2}$ and $P < 0.01$, $F_{1,13} = 12.51$ for f_H; Fig. 4, *A* and *B*), and the relative increases were 109% and 51%, respectively. There was a trend for a change in the relative increase in $\dot{V}_{O_2}$ between *tests A* and *B* ($P < 0.1$, $F_{1,4} = 3.67$) from 74% to 144%. There was a significant decrease in $\dot{V}_{O_2}$ and f_H in air from *test A* to *test B* ($P < 0.05$, $F_{1,13} = 5.16$ for $\dot{V}_{O_2}$ and $P < 0.01$, $F_{1,13} = 8.56$ for f_H). OP increased ($P < 0.01$, $F_{1,14} = 80.1$) by ~40% (from 1.12 to 1.47 ml O_2/heartbeat) in water compared with air (Fig. 4*C*).

DISCUSSION

The main findings from this study are as follows: *1*) $\dot{V}_{O_2}$ decreased throughout the fasting period, and this decrease was mainly explained by changes in M_b, *2*) there was a change in the $\dot{V}_{O_2}$-f_H relationship in exercising birds during fasting but not during rest, *3*) the duration of fasting was a satisfactory proxy in a predictive equation for all the factors that influence the $\dot{V}_{O_2}$-f_H relationship during the fast, and *4*) $\dot{V}_{O_2}$, f_H, and OP increased significantly from air to water at 4°C.

M_b Loss

M_b decreased by 30.7 ± 2.7% (range 27.1–34.1%) from *test 1* to *test 5*, and the $dM_b/M_b \cdot dt$ data (Fig. 1*B*) indicate that, except perhaps for *test 1*, all experiments in this study were performed during phase II of fasting. The fact that the rate of loss of M_b between *tests 1* and *2* in the present study mirrored that of fasting animals in the colony before egg laying (17) suggests that the initial rapid loss in M_b was not caused by stress of being confined to an enclosure and handling.

Resting Values During Treadmill Experiments

$\dot{V}_{O_2}$. The range of mean resting $\dot{V}_{O_2}$ values observed if birds and tests were pooled (164.7 to 58.5 ml O_2/min) was similar to values obtained by others during shorter [14–17 days, 152.1 to 112.5 ml O_2/min (15)] or similar [30 days, 169.8 to 74.9 ml O_2/min (26)] fasting durations. There was a much greater proportional decrease in resting $\dot{V}_{O_2}$ than in M_b (Tables 1 and 3). As a result of these changes, the mass exponent for $\dot{V}_{O_2}$ for rest only was 1.89. In a previous study, the magnitude of change in $\dot{V}_{O_2}$ between arriving and departing males was 29.7%, whereas the change in M_b was 11.5% (15).

Intraspecific allometric exponents >1 have been observed in different situations in birds with large individual variation in M_b, for example, in the kestrel (*Falco tinnunculus*) fed a hypocaloric diet (12), in the European barn owl (*Tyto alba alba*) during refeeding after starvation (21), and in fasted emperor penguins (13). In the first case, it was suggested that the reduction in $\dot{V}_{O_2}$ with decreasing M_b led to a decrease in body temperature. We hypothesize, therefore, that there is a decrease in body temperature during fasting in king penguins, but this is yet to be tested.

The fact that the allometric exponent for $\dot{V}_{O_2}$ was >1 for all tests in the present study suggests that during fasting the resting metabolic rate decreases below the basal metabolic rate of postabsorptive birds, a situation of hypometabolism observed during the incubation fast in the emperor penguin (1). It is clear from the data of Cherel et al. (8) that, during phase II of fasting, there is a greater proportional loss of highly metabolically active organs such as the liver (11); however, it is also clear that the rate of reduction in lean body mass is proportionately slower than that of whole body mass. Thus the proportionately greater loss of the most metabolically active organs cannot, in itself, explain the high mass exponent for $\dot{V}_{O_2}$. Other possibilities are channel arrest, differential gene expression, and regulatory changes in the protein synthesis and degradation (32). The proportional change in $\dot{V}_{O_2}$ between *test 2* and *test 4* in the present study was 26.2%, which was similar to that reported previously for fasting king penguins (29.7%) for a comparable fasting duration (15.5 days) (15).

f_H. The resting f_H decreased throughout the fasting period (Fig. 2*B*), and the range of mean resting f_H values for the first 13.4 days of fasting (*tests 1–3*, Table 3) were 87.6–92.9 beats/min, which is similar to the values previously reported for arriving and departing males (15).

Resting Values in Air and Water Experiments

$\dot{V}_{O_2}$ *in air*. The ranges of mean resting $\dot{V}_{O_2}$ values for birds in air during *tests A* and *B* were similar to those from birds with similar fasting periods during the treadmill tests. This indicates

that both protocols gave equivalent values. In addition, the fact that $\dot{V}_{O_2}$ was not different in 4°C air or during rest on the treadmill at 10.1 to −19.8°C shows that the penguins were in their thermoneutral zone. This agrees with an earlier study in which the lower critical temperature for king penguins in air was approximately −5°C (16).

$\dot{V}_{O_2}$ and f_H in water. Mean $\dot{V}_{O_2}$ for birds in water during *tests A and B* was 172.3 ml O₂/min, which is similar to that previously reported for king penguins resting at 9°C (160 ml O₂/min) (10) or at 5°C (182.9 ml O₂/min) (25) at similar M_b values. The mean resting $\dot{V}_{O_2}$ values in water at 4°C for *tests A and B* (168.8 and 175.9 ml O₂/min, respectively) were 74% and 144% higher, respectively, than for animals in air (98.0 and 72.8 ml O₂/min, respectively), which are similar to the differences measured at 10°C in the little penguin (~80–100%, *Eudyptula minor*) (30), the gentoo penguin (~88%, *Pygoscelis papua*) (3), and eider ducks (140%, *Somateria mollissima*) (22). The f_H was 51% higher in water than in air. This difference is higher than the increase measured in Humboldt penguins (15%, *Spheniscus humboldti*) (6) or eider ducks (40%) (22) but lower than the difference in gentoo penguins (~70%) (3).

Exercise Values During Treadmill Experiments

$\dot{V}_{O_2}$ during exercise. The highest speed used throughout the present study and achieved by all animals was 1.8 km/h, even though it has been shown that some king penguins are able to sustain speeds of up to 2.5 km/h (15). To be able to use the f_H technique in the field, it is important during the calibration studies to cover a range of f_H values that are similar to those observed in the field. In the field, <1% of f_H values ashore exceeded 130 beats/min (G. Froget et al., unpublished observation). Consequently, in the present study, an appropriate range of f_H values was achieved, although there was no attempt to exercise the birds at intensities approaching their maximum $\dot{V}_{O_2}$.

f_H during exercise. During *tests 1 and 5*, when the birds were walking at 0.3 km/h, f_H increased by 45% and 132%, respectively, above the resting values. The f_H increased by a further 56% and 32% for *tests 1 and 5*, respectively, as the speed increased from 0.3 to 1.8 km/h.

Although the maximum value of f_H for *test 1* in the present study was significantly different (Table 3, 212.1 ± 29.4 beats/min) from that of arriving males ($P < 0.05$, $t_{0.05,12} = 2.56$, 148.8 ± 50.1 beats/min), maximum f_H for *test 3* (226.2 ± 58.9 beats/min) was not significantly different from that of departing males ($P > 0.3$, $t_{0.05,12} = 0.76$, 196.6 ± 73.2 beats/min, $n = 9$) in a previous study (see Table 2 in Ref. 15).

$\dot{V}_{O_2}$-f_H Relationship

Fasting-related changes. The best multivariate model including U, M_b, and t as covariates suggested that the $\dot{V}_{O_2}$-f_H relationship in the present experiments was affected by the activity level and a complex effect of fasting on M_b and the body composition that warranted inclusion of t. Thus the results in this study support the hypothesis of a change in the $\dot{V}_{O_2}$-f_H relationship with extended fasting during exercise but not at rest. These findings are in agreement with those in the study by Froget et al. (15), where it was concluded that the relationship changed with fasting in males. However, conclu-

sions from the results of Froget et al. need to be made with caution, as neither the length of fasting nor M_b was considered during the analysis of these data. Also, in the previous study, the fasting durations were different between males and females, which could explain the difference between the genders (15). Indeed, a new analysis of these previous data, including M_b and t, indicated that there were no differences between gender.

Possible explanations for the small mass-independent changes in the $\dot{V}_{O_2}$-f_H relationship during fasting were postulated by Froget et al. (15) and include changes in any of the variables in the term $V_s \cdot (Ca_{O_2} - C\bar{v}_{O_2})$, i.e., the OP, of the Fick equation, where V_s is the cardiac stroke volume and Ca_{O_2} and $C\bar{v}_{O_2}$ are arterial and mixed venous O₂ concentrations, respectively (4). Thus fasting may cause a decrease in O₂ extraction at the tissue level and/or a decrease in V_s throughout the fast (15), which could be particularly significant during elevated levels of exercise. A reduction in the capillary density during fasting may be the result of prolonged inactivity during the fasting period, which in turn results in a reduced O₂ extraction.

Predicting $\dot{V}_{O_2}$ in the field. A study of king penguins during their breeding period reveals two distinct field situations: *1)* fasting on shore while molting, incubating, or rearing a chick and *2)* foraging at sea. In both cases, an equation including M_b and speed is not practical for estimation of the $\dot{V}_{O_2}$ of an animal but is only appropriate in a laboratory setting, where several of the confounding variables can be estimated and properly controlled. The variable t and *Eq. 1* may be useful only during terrestrial fasting periods when the $\dot{V}_{O_2}$-f_H relationship changes. As the animal shifts from its time ashore and enters the sea, fasting may persist for only another 1–2 days as the bird travels to the foraging site. Thereafter, the animal continuously consumes prey during most of the foraging trip, and the use of the variable t may be inappropriate. Furthermore, the physiological state of the birds while they are at sea may be close to that immediately after they return to the colony, i.e., that of being fully fed (*test 1*). Consequently, when the data for *test 1* only were used, there was no significant difference in the slopes ($F_{4,20} = 2.04$, $P > 0.1$) between animals but a significant difference in the intercepts ($F_{4,24} = 3.10$, $P < 0.05$). The model with a random effect for the intercept resulted in the following equations

$$\log(\dot{V}_{O_2}) = -0.488 + 1.35 \cdot \log(f_H) \quad (r^2 = 0.86) \quad (2a)$$

and

$$\log(\dot{V}_{O_2}) = -11.1 + 11.3 \cdot \log(f_H) \\ - 2.33 \cdot \log(f_H)^2 \quad (r^2 = 0.91) \quad (2b)$$

It is, of course, possible that the reversal in the $\dot{V}_{O_2}$-f_H relationship takes place over an extended period during refeeding. Thus it is necessary to determine the length of time required for the birds to return to the fully fed state after their return to sea.

While the birds were on shore, even though M_b was important in defining the physiological changes due to fasting, it explained only an additional 2% of the variation of the data compared with the model including all the important covariates. The high correlation between M_b and t (Fig. 1B) allowed most of this additional variation to be explained by t alone. In addition, inclusion of an interaction term allowed the regression model to predict $\dot{V}_{O_2}$ satisfactorily after removal of speed

(*Eq. 1*). Consequently, *Eq. 1* could be used to estimate the energetics during the molting, courtship, and incubation periods on land. The estimated $\dot{V}_{O_2}$ from 10 breeding king penguins (G. Froget et al., unpublished observation) was significantly lower when *Eq. 1* was used (112.2 ml O_2/min, $P < 0.05$, Z-test) than when either *Eq. 2A* (146.9 ml O_2/min) or the equation developed by Froget et al. (15) ($\dot{V}_{O_2} = 3.39 \cdot f_H - 136.86$, 158.5 ml O_2/min) was used. This emphasizes the importance of including t when estimating field $\dot{V}_{O_2}$ in penguins when they are ashore.

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