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Metabolism and thermoregulation during fasting in king penguins, *Aptenodytes patagonicus*, in air and water

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Fahlman, A., A. Schmidt, Y. Handrich, A. J. Woakes, and P. J. Butler. Metabolism and thermoregulation during fasting in king penguins, *Aptenodytes patagonicus*, in air and water. *Am J Physiol Regul Integr Comp Physiol* 289: R670–R679, 2005. First published May 12, 2005; doi:10.1152/ajpregu.00130.2005.—We measured oxygen consumption rate ($\dot{V}O_2$) and body temperatures in 10 king penguins in air and water. $\dot{V}O_2$ was measured during rest and at submaximal and maximal exercise before (fed) and after (fasted) an average fasting duration of 14.4 ± 2.3 days (mean \pm 1 SD, range 10–19 days) in air and water. Concurrently, we measured subcutaneous temperature and temperature of the upper (heart and liver), middle (stomach) and lower (intestine) abdomen. The mean body mass (M_b) was 13.8 ± 1.2 kg in fed and 11.0 ± 0.6 kg in fasted birds. After fasting, resting $\dot{V}O_2$ was 93% higher in water than in air (air: 86.9 ± 8.8 ml/min; water: 167.3 ± 36.7 ml/min, $P < 0.01$), while there was no difference in resting $\dot{V}O_2$ between air and water in fed animals (air: 117.1 ± 20.0 ml O_2 /min; water: 114.8 ± 32.7 ml O_2 /min, $P > 0.6$). In air, $\dot{V}O_2$ decreased with M_b , while it increased with M_b in water. Body temperature did not change with fasting in air, whereas in water, there were complex changes in the peripheral body temperatures. These latter changes may, therefore, be indicative of a loss in body insulation and of variations in peripheral perfusion. Four animals were given a single meal after fasting and the temperature changes were partly reversed 24 h after refeeding in all body regions except the subcutaneous, indicating a rapid reversal to a prefasting state where body heat loss is minimal. The data emphasize the importance in considering nutritional status when studying king penguins and that the fasting-related physiological changes diverge in air and water.

thermoregulatory plasticity; hypometabolism; sea bird; allometry

THE USE OF HEART RATE (f_H) as an indicator of the oxygen consumption rate ($\dot{V}O_2$) has previously been used to estimate field metabolic rate in king penguins both on land and in water (21). Unfortunately, the relationship between f_H and $\dot{V}O_2$ for a given species does not necessarily remain constant throughout the life history. The relationship has been shown to vary with the type of activity (7, 43), physiological state (fasting, breeding; 17, 20), and with season (28). Whereas the relationship was shown to be similar in air and water in gentoo (3) and macaroni penguins (24), the use of f_H to predict $\dot{V}O_2$ in other penguin species requires validation studies to be performed both in water and on land.

Before attempting to estimate the relationship between f_H and $\dot{V}O_2$ in king penguins in water, we considered it crucial first to study the complex body temperature changes (thermoregulatory plasticity) reported in this species (26). This is important for two reasons. First, we previously measured a significant reduction in $\dot{V}O_2$ during fasting in air and hypothesized that this was in part due to a change in the body temperature of the birds (17). This observation prompted us to try to determine whether similar changes occur in king penguins while fasting in water. Secondly, as $\dot{V}O_2$ decreases during fasting in air, a rapid reversal of this reduction after refeeding would be indicative of physiological or biochemical adjustments, while a prolonged reversal could be indicative of changes in morphology, e.g., increased subcutaneous fat layer. In addition, the thermally challenging exposure to sea water is of considerable interest. It is well recognized that heat loss in water is greater than that in air at the same temperature and that this stems from the greater specific heat and heat conductivity of water than those of air (5, 22). Because of the complexity of heat loss processes in water and air, this generalization has only limited value. Hence, measuring regional temperature responses concurrently with measurements of metabolic rates in air and water could significantly improve our understanding of the thermoregulatory plasticity observed in king penguins.

Increased metabolic rate is commonly observed in birds and mammals during submergence in water as a response to the increased heat loss. Heat loss can be reduced to a minimum by increasing peripheral insulation, and this can be achieved either by increasing the thickness of the subcutaneous fat layer and/or by decreasing blood flow, and hence heat flow, between the body core and the periphery (5). Thus the thermal insulation of diving birds and mammals is believed to be directly related to the amount of subcutaneous fat and/or the peripheral perfusion (39), and in birds, additional insulation is provided by the air trapped in the feathers (30). A better understanding of the physiological responses of penguins in water is important to understand the energetic cost for these animals while at sea. Therefore, the main objective of the present study was to measure total $\dot{V}O_2$ and differences in temperature between different regions of the body in king penguins both in air and water.

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MATERIALS AND METHODS

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

Animals and Experiments

The experiments were carried out on Possession Island (Crozet Archipelago 46°25' S, 51°45' E) over the Austral summer of 2003–2004. Ten courting male king penguins, *Aptenodytes patagonicus*, were used for the experiments. Gender was determined by the song of each individual (29) and later confirmed by genetic analysis (Avian Biotech International, Truro, Cornwall). All birds were caught on the beach, near the breeding site at the earliest stage of courtship and just after their arrival in the colony in late December. At this stage in the courtship, mate choice is not yet made. The birds were caught in the afternoon and immediately weighed. Each animal was fitted with a temporary plastic flipper band for recognition and placed in a wooden enclosure (size 3 m × 3 m) where they were kept for the duration of the fasting periods. Only birds with an initial mass > 13.0 kg were used in the experiments, a body mass (M_b) known to allow male king penguins to fast for at least 1 mo while incubating the egg (23). In addition, each bird underwent an initial test in the water channel to determine its behavioral response in water. Only those birds that appeared calm and exercised well, that is, swam under water, in the channel were chosen (10 out of 14 captured).

Animals

One to four days (2.5 ± 1.2 days, mean \pm SD) after capture, each bird underwent surgery for implantation of a data logger (DL; see *Surgical Protocol* below) (45), which measured the temperature of the upper, middle, and lower abdomen (DL_{abd}) (see Fig. 1). The middle temperature was measured by a temperature sensor in the logging unit, while two thermistor leads, covered by a silicone sleeve, were each tunneled in opposite directions to measure the temperatures of the upper and lower abdomen. The upper abdominal thermistor was located close to the heart; the middle abdominal thermistor was placed immediately beneath the breastbone; and the lower abdominal thermistor was situated at the lower end of the brood patch. Eight birds among the ten also had a temperature logger implanted subcutaneously above the leg. The thermistor was located inside the logger and the logger placed on the lateral aspect of the midaxillary line, immediately above the leg, thus measuring the temperature between the subcutaneous fat and the underlying muscle (DL_{leg}). Nine of the 10 DL_{abd} and 7 of the 8 DL_{leg} were retrieved with data on them.

During the surgery, a yellow picric acid mark was painted on the chest to aid identification of the bird. In addition a fish tag, which consisted of a clear and a colored end, was placed on the back of each bird. The clear end was placed subcutaneously using a sterilized needle while the colored piece was protruding the skin but laying flat against the feathers. Having both ventral and dorsal markings enhanced the possibility of detection from a distance. Surgical recovery was ensured by allowing each animal to rest for the next 10.8 ± 1.4 days (range 9–13 days) in the wooden enclosure without human intervention, except when weighing the animal. Following the recovery period, which was longer than that known for animals to revert to normal behavior after surgery (21), each bird was placed on a treadmill (exercise in air) and in a water channel (exercise in water). These experiments were, therefore, performed on fasting birds, and after this initial set of experiments the birds were released on the beach close to where they had been caught.

No animal was allowed to fast to below its critical body mass (cM_b), a value dependent on body size and already estimated in this

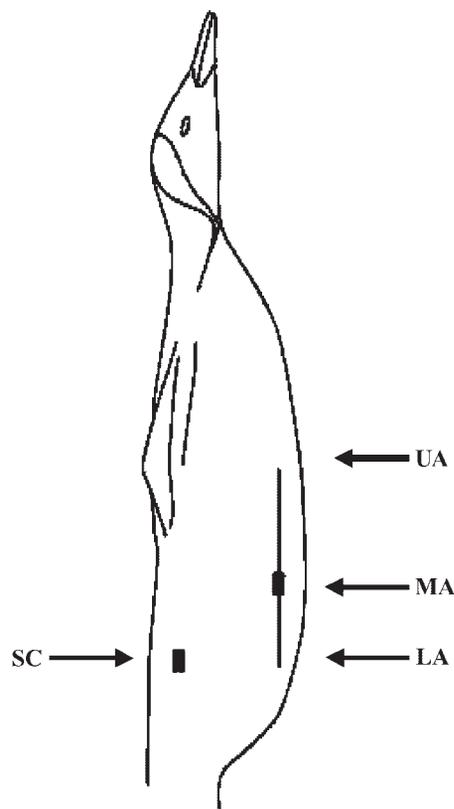


Fig. 1. Side view of a king penguin showing the placement of loggers. Thermal sensor probes were placed in the upper (UA), middle (MA, sensor placed in logger), and lower abdomen (LA). The subcutaneous (SC) sensor was located in the logger, which was placed in the flank of the bird.

species (23). Upon release, the birds did not initiate a new session of courtship but went to sea to replenish their body reserve for a new attempt to breed (23). All 10 birds were recaptured after they returned from the foraging trip, between 13 and 25 days later, in the same area where they had been released. Within the next two days, each bird was again placed in the water channel or on the treadmill. Thus these experiments were performed on (recently) fed birds. The loggers were removed after the end of this second set of water channel and treadmill experiments in 6 out of the 10 birds. The remaining 4 birds were again placed in the wooden enclosure and fasted a second time for an average of 17.5 ± 1.3 days (range 16–19 days).

These four birds were fasted and tested again (*fasting II* experiment). After the *fasting II* experiment, each bird was fed an average of 920 ± 72 g of sprat, and the animal was returned to the enclosure for ~ 24 h. Next, the bird was again placed in the water channel and on the treadmill for a fourth set of experiments (refeeding experiment). Three of these birds had a DL_{leg} and a DL_{abd} implanted, while the fourth only had a DL_{abd}. After the fourth experiment, the loggers were removed. There was no difference in the duration of the two successive fasting periods in the 4 birds ($P > 0.1$, two-tailed *t*-test, Table 1).

After removal of the loggers, all of the 10 birds were observed for two days while kept in the enclosure and then released into the wild on the beach where they were initially captured. Throughout the experimental procedure, M_b of each bird was measured every 1 to 2 days to determine the fasting phase from the mass-specific daily loss in body mass as previously detailed ($dM_b/M_b \cdot dt$, $g \cdot kg^{-1} \cdot day^{-1}$; Refs. 17 and 32).

Table 1. Summary morphometrics for 10 male king penguins used to determine fasting-related changes in \dot{V}_{O_2} while in air and while in water

Bird ID	M_b of Birds Returning From Sea (Fed Birds), kg		M_b at End of First Fasting Period, kg		Duration of First Fasting Period, days		Duration Between Last Dive and Fed Experiment, days		Feeding Duration, days	Duration of Second Fasting Period, days	M_b at End of Second Fasting Period, kg	cM_b , kg
	Air	Water	Air	Water	Air	Water	Air	Water				
A	14.07	14.22	11.10	10.70	13	15	2	1	25	18	11.19	8.94
B	13.06	12.50	10.83	10.30	13	15	1	2	22	—	—	9.42
C	13.97	13.67	11.20	10.90	14	16	1	1	15	—	—	9.79
D	15.11	14.70	10.70	10.98	19	17	1	1	13	—	—	9.48
E	13.14	13.01	10.80	10.53	10	12	1	1	18	—	—	9.23
F	13.61	13.87	11.24	10.63	15	14	2	1	19	—	—	9.47
G	11.79	11.96	10.60	10.84	14	12	2	1	23	—	—	8.79
H	14.94	14.61	11.20	10.97	15	16	1	2	23	19	12.33	9.78
I	16.22	15.94	12.67	12.46	17	17	1	2	18	16	13.21	10.43
J	12.99	13.35	10.90	11.03	12	11	2	0	20	17	11.04	9.21
\bar{x}	13.89±1.27	13.78±1.16	11.12±0.59	10.93±0.58	14.2±2.5	14.5±2.2	1.3±0.5	1.2±0.6	19.6±3.8	17.5±1.3	11.9±1.02	9.45±0.47
<i>P</i> value	> 0.3		> 0.05		> 0.6		> 0.7		> 0.1		> 0.1	

M_b , body mass; cM_b , critical body mass (23). Duration of first fasting period is the number of days from capture until the experiment was conducted. Duration between last dive and fed experiment is the number of days between the last dive to 70 m until the experiment. Feeding duration is the number of days between release until capture. \bar{x} is the grand mean (\pm SD) for each variable. *P* values were generated from paired *t*-tests between mean values for the two experimental conditions (in air vs. in water) or for the 1st and 2nd fasting M_b or duration.

Surgical Protocol

The surgical procedure used has already been described in detail by Froget et al. (21), but with the following modifications. Anesthesia was induced using isoflurane (Aerrane, Baxter Health Care, Thetford, UK) in O_2 delivered through a plastic hood placed over the head of the bird. Lactated Ringer solution (\sim 50 ml/h, Laboratoire Aguettant, Lyon, France) was administered intravenously over the course of the surgery at a rate of \sim 50 ml/h. The incisions were sutured closed, and the animal was given intramuscular injections of ketofen (2 mg/kg, Merial, Lyon, France) and terramycin (1 mg/kg, Long-acting T.L.A., Pfizer, France) to inhibit postoperative infection. Secondary injections of ketofen and terramycin were administered postsurgically at 24 and 48 h, respectively. The bird was kept isolated in a wooden enclosure until it had fully recovered from the anesthetic (\sim 1–2 h) and then returned to the common enclosure, which housed the other birds. The same surgical and recovery procedures were used during the removal of the loggers.

A 0.3-ml blood sample from the brachial flipper vein was taken for gender determination by genetic analysis (Avian Biotech International, Truro, Cornwall, UK) and the length of the flipper was measured allowing determination of cM_b .

Experimental protocol and respirometry. A set of experiments included both a treadmill (exercise in air) and a water channel test (exercise in water). Each of the 10 birds repeated a set of experiments twice, while the refed group of 4 birds also conducted a third and a fourth set immediately before and 24 h after refeeding. For each set of experiments, the water channel and treadmill experiments were separated at most by 2 days, and the order was randomized between birds. The M_b , (kg) was determined for each bird before each experiment (Table 1).

Exercise in air. Treadmill experiments were conducted as previously detailed (17). In short, the bird was placed in the respirometer (80 × 46 × 86 cm; length × width × height) and allowed to rest for \geq 60 min. The final 5 min of stable readings during this period was considered to be the resting metabolic rate in air (RMR_{air}). Next, each bird walked at one of five different speeds (0.3, 0.7, 1.0, 1.5, and 1.8 km/h). The sequence of walking speeds was assigned at random for each bird, but the sequence of speeds was the same for each bird between experiments. The animal walked at each speed until steady values for \dot{V}_{O_2} and \dot{V}_{CO_2} were obtained for at least 5 min, which was usually after 12–17 min of walking. Therefore, a walking session usually lasted between 17 and 22 min. Each walking session was

separated by a period of rest until \dot{V}_{O_2} and \dot{V}_{CO_2} had reached stable values similar to those recorded during the initial 60-min resting period. The rest period lasted for at least 30 min.

Exercise in water. A static water channel (30.0 × 1.4 × 1.2 m; length × width × height) was used. Underneath the wooden cover a plastic mesh was submerged \sim 5 cm under the water to deny the animal access to air along the length of the water channel. Doors were placed in the wooden cover every 3 m to allow easy access to the inside of the channel. At each end of the water channel, a clear plastic respirometer box was submerged \sim 5 cm into the water through a hole in the wooden cover. The air space of each respirometer box measured \sim 89 × 39 × 16 cm (length × width × height), and this size was sufficiently large to allow the animal to turn and to rest without restriction. Inside each box, there were two fans attached to its upper surface, thus producing rapid mixing of the internal gases. The experiment began by placing the bird in one of the openings to the water channel and the respirometer box was then placed over the opening. Data collection began 1 min after the animal had been placed in the respirometer box and continued until the end of the experiment. The animals were left in the respirometer box for an average of 179 ± 29 min ($n = 20$, 10 birds and 2 experiments) and allowed to behave freely.

Observations were made continuously without intervention, except for times when the respirometer boxes were covered to tempt active birds to rest for a period of time. Most animals were agitated when initially placed in the box and often made what appeared to be attempts to leave the box, but they all settled within 1–3 min, after which most began to swim underwater. Their activity in the water channel was variable, with some animals swimming for almost the entire experimental period while others only swam under water a limited number of times. Nevertheless, four distinct behaviors were observed for all animals; resting, preening, searching, and swimming. “Resting” included only those periods when the animal was completely still. “Searching” included periods when the animal inspected the respirometer box or short dives of < 15 s when the animal did not leave the area of the respirometer box. “Swimming” included all periods of exercise > 15 s and underwater travel between the respirometer boxes located at each end of the water channel. “Preening” was usually seen during periods of rest when the animal actively cleaned its feathers.

Respirometry. The \dot{V}_{O_2} and \dot{V}_{CO_2} for the treadmill and water channel experiments were measured by a common recording system,

which could be switched to sample gas from either respirometry chamber. The system was built as a flow-through respirometer system similar to that used by Fahlman et al. (17) with the following modifications. In the water channel, the gas flow from the two respirometer boxes was joined to a common hose. This assured that the excurrent gas from the two boxes was properly mixed. The excurrent flow-rate of the treadmill and water channel respirometers were ~ 107 l/min and ~ 120 l/min, respectively, as measured by a flowmeter (KDG 100, KDG Instruments, Sussex, England). A subsample of this gas was 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 from a commercial mixture (Messer, Asnieres, France).

Temperature, humidity, and ambient pressure inside and outside of the respirometer boxes were measured using suitable sensors (Farnell Electronics) and ranged between 5 and 20.8°C, 47.5 and 100%, and 99.9 and 102.6 kPa for the treadmill experiments and between 7.8 and 22.3°C, 39.5 and 100%, and 99.9 and 102.9 kPa for the water channel experiments. Mean air temperatures inside the respirometers were $13.9 \pm 2.0^\circ\text{C}$ and $15.7 \pm 2.1^\circ\text{C}$ for the treadmill and water channel, respectively. Mean water temperatures were $8.6 \pm 0.6^\circ\text{C}$ and $8.8 \pm 0.7^\circ\text{C}$ for the fed and fasting experiment, and mean water temperatures for the second fasting and refeeding experiments for the four birds fasted a second time were 8.9 ± 0.3 and $8.8 \pm 0.3^\circ\text{C}$, respectively.

The accuracy of both respirometry systems was determined by simultaneous N_2 -dilution and CO_2 -addition tests (19), and these showed that the difference between the observed and expected values were within 4% for both the treadmill and the water channel respirometry systems, confirming that the systems were properly sealed. The leak test for the treadmill respirometer was unaffected by the treadmill speed. The CO_2 -addition test confirmed that minimal amounts of CO_2 were lost by dissolving in the seawater in the water channel. The time constants of the treadmill and water channel respirometry systems were ~ 3 min and ~ 1.2 min, respectively, including the volume of the respirometer and the plastic hose to the analyzers. The time required to reach a 95% fractional transformation to a new steady state can be computed as 3.2 times the time constant, or ~ 9.5 min for the treadmill respirometer system and ~ 3.5 min for the water channel respirometer system. In the water channel, there was no difference in the time constant between respirometer boxes.

Data were sampled and saved as previously described (17). All flows were corrected to standard temperature (273K) and pressure (101.3 kPa) dry (STPD).

Heat Loss

The thermal conductance (C , $\text{W}\cdot\text{m}^{-2}\cdot^\circ\text{C}^{-1}$) of each bird was calculated as (5, 31):

$$C = \frac{\dot{M} \pm h_{r+e} \pm S}{(T_b - T_w) \cdot SA} \quad (1)$$

where \dot{M} (W) was estimated from the $\dot{V}\text{O}_2$ assuming that 1 ml $\text{O}_2\cdot\text{s}^{-1} = 19.8$ W (21), T_b and T_w ($^\circ\text{C}$) were the body (upper abdominal) and water temperatures, respectively, and SA the surface area (m^2) as described by Pinshow et al. (36) for the emperor penguin ($SA = 0.065 \cdot M_b^{-0.667}$). The upper abdominal temperature was chosen as the best representation of deep body core temperature, as it is the area where several major organs such as the liver, heart, and pectoral muscle, are located. It can be expected that the animal would not lose any heat by evaporation (h_e) from the body surface when submerged (22). In addition, the respiratory heat loss (h_r) has been estimated to be negligible compared with the total heat loss in air in small mammals (18) and in Adélie penguins (8). Therefore, the respiratory and evaporative heat transfer rates were assumed to be

negligible in the water channel ($h_{r+e} = 0$). The heat stores (S) are difficult to estimate, especially during periods of rapid changes in body temperature. However, S is zero when the animal is in thermal equilibrium. In the current study, the upper abdominal temperature reached equilibrium ($S = 0$) after the animal had been in the water channel for 20 min (Fig. 2A). Then, from this time, the thermal conductance could be estimated. Therefore, we estimated the conductance every 30 min in the water channel, which omitted the period of changing S .

Data Assessment and Statistical Analysis

All values are reported as means \pm SD, unless otherwise specified. Student's t -test was used to compare the difference between the means of two populations. ANOVA with 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, for example, log-transformation. In the case of unequal variances, Mann-Whitney or Kruskal-Wallis statistical tests were used. We utilized mixed models regression, using a compound symmetry covariance structure to deal with the correlation within animals (SAS, version 8; 33). Statistical significance was set at $P < 0.05$ level, while $0.05 < P < 0.1$ was considered a trend (17).

Oxygen consumption and carbon dioxide production rates were calculated using standard equations (15, 44), as described in Froget et al. (20). The average $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were estimated from the gas concentrations during the last 2 min at each speed on the treadmill. For the water channel experiments, $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were averaged over 5-min periods for the whole experimental period.

For the current study, only the data for RMR, submaximal and maximal exercise, was used for birds in air and water. Submaximal were exercise metabolic rate in air was considered the metabolic rate at a speed of 1.0 km/h. Resting metabolic rate in water ($\text{RMR}_{\text{water}}$) was considered to be the resting $\dot{V}\text{O}_2$ after ≥ 20 min of continuous rest. Submaximal metabolic rate in water was the median $\dot{V}\text{O}_2$ for all 5-min periods during the entire experiment. Maximal metabolic rate was that recorded at the highest treadmill speed or that during the 5-min period in the water channel that gave the highest $\dot{V}\text{O}_2$.

RESULTS

Morphological summary statistics and the total number of days fasting are presented in Table 1, for the 10 birds.

Body Mass During Fasting

The body mass loss throughout both fasting periods for the 10 king penguins in the current study were similar to those from a previous study (17). The mass specific rate of change in body mass ($dM_b/M_b \cdot dt$) remained more or less constant beyond day 5 of the fasting period ($14.4 \pm 2.4 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), and no bird showed an increase in $dM_b/M_b \cdot dt$ before their release. This indicated that no animal entered *phase III* of fasting (25, 32), which is associated with a signal to abandon the egg and refeed in the free-ranging bird.

The 10 animals lost an average of 20% (from 13.82 to 11.03 kg, mean of values from experiments in both air and water) of their M_b during the fasting period (Table 1). The four animals that performed two fasting periods lost an average of 19% (from 14.04 to 11.38 kg) and 14% (from 13.84 to 11.94 kg) of their M_b for their first and second fasting periods, respectively (Table 1). During their foraging trip, the birds had gained average mass of 3.58 ± 1.12 kg (range 1.91–5.28 kg). They were caught at their second arrival on the colony which was, on

average, 13.2 ± 16.6 h (range 0.5–42 h) after their last dive during daylight hours to more than 70 m. This was assumed to be their last feeding dive (9, 37).

Exercise in Air

The \dot{V}_{O_2} at each speed was similar to those previously reported (17). Mean \dot{V}_{O_2} at rest, submaximal (1 km/h speed) and maximal exercise (1.5 or 1.8 km/h speed) are summarized in Table 2. RMR_{air} decreased during fasting in all animals (mean 26%, range 1–44%, Table 2). Mean \dot{V}_{O_2} at submaximal and maximal exercise for the 10 birds decreased during fasting by 15% and 20%, respectively. There were a few exceptions in exercising birds, and the \dot{V}_{O_2} increased with fasting in 3 and 2 animals at submaximal (range 20% to 36%) and maximal exercise (range 6% to 38%), respectively. There was no change in either body temperature with fasting ($P > 0.1$, paired *t*-test), and the mean (\pm SD) upper abdominal ($n = 7$) and subcutaneous ($n = 8$) temperatures were $38.6^\circ\text{C} \pm 0.9$, $38.9^\circ\text{C} \pm 0.5$ before and $38.4^\circ\text{C} \pm 0.8$ and $38.5^\circ\text{C} \pm 1.5$ after fasting, respectively.

Exercise in Water

In the water channel, the percentage of time spent at rest, preening, searching, and swimming was 54.4%, 6.2%, 22.8%, and 16.6%, respectively, for fasting birds and 73.9%, 1.0%, 14.0%, and 11.1%, respectively, for fed animals. This does not include data from the second fast or the refeeding experiments.

In contrast to the observations in air, there was a significant increase in the \dot{V}_{O_2} in water ($\dot{V}_{O_{2water}}$) with fasting at rest (46%), and during submaximal (33%) and maximal exercise (16%, Table 2) in water. Except for one bird at rest, $\dot{V}_{O_{2water}}$ increased systematically in all birds and during all activities with fasting. The bird with a decrease in RMR_{water} with fasting (–20%) was never seen to rest for >10 min during the fed experiment. Therefore, resting \dot{V}_{O_2} of this bird was higher than those of the remaining birds, which may explain this single discrepancy. Even though the relative increase in $\dot{V}_{O_{2water}}$ with fasting decreased with activity, from 46 to 16%, the absolute increase in $\dot{V}_{O_{2water}}$ was more or less constant among activities, ranging between 48.7 and 59.0 ml O_2 /min (Table 2).

For the following comparison, it must be emphasized that, other than comparison between RMR values in air and water, the comparisons between maximal and submaximal exercise in water and air depend critically on our definition, and any conclusions should be made with care. In animals that returned from the sea (fed experiments), there was no difference in

either resting \dot{V}_{O_2} , or in \dot{V}_{O_2} at maximal exercise in water compared with those in air, but at submaximal exercise, the values were significantly lower in water compared with those in air (Table 2). After fasting, on the other hand, the $\dot{V}_{O_{2water}}$ were 93% and 23% higher at rest and at maximal exercise, respectively, compared with those in air (Table 2).

In water, the mass specific \dot{V}_{O_2} ($s\dot{V}_{O_2}$, ml $O_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) at rest, and at submaximal, and maximal exercise increased by 85%, 68%, and 46%, respectively, with fasting. The mass exponent (*b*) was determined by the classical allometric equation $\log(\dot{V}_{O_2}) = a + b \cdot \log(M_b)$ (38). In fed birds, there was no relationship between $\log(M_b)$ and $\log(\dot{V}_{O_{2water}})$ ($P > 0.1$), whereas in fasting birds, there was an inverse relationship with a mass exponent of -2.72 ($P < 0.05$). Combining the $\dot{V}_{O_{2water}}$ data for fed and fasting birds, there was a trend for a change in the mass exponents with activity ($F_{2,44} = 2.78$, $P < 0.1$). At rest, the allometric mass exponent in water was -1.45 , while during submaximal and maximal exercise, they were -1.15 and -0.34 , respectively ($P < 0.01$, mixed-model repeated-measures ANOVA).

Fasting did not change any of the body temperatures while the birds were still in air (i.e., time 0, Fig. 2, A–D, all $P > 0.1$, paired *t*-test). As the animal entered the water, body temperatures rapidly decreased to new steady values, which were usually achieved between 20–60 min (Figs. 2, A–D), in both fed and fasted birds. There was no difference in upper abdominal temperatures between fed and fasted birds (Fig. 2A), but the middle and lower abdominal temperatures and the subcutaneous temperature were significantly lower in fasting birds after 80 min (Fig. 2B) and 60 min (Fig. 2, C and D, $P < 0.05$), respectively. The maximum and mean changes in each body temperature were calculated as the preexperimental body temperature minus the minimum or mean temperature for the whole experimental period. For the upper, middle, and lower abdominal temperatures, there were significant changes with fasting in both the maximum and mean change in temperature during a water channel experiment (Table 3). The temperature difference between the upper and lower abdominal temperature was significantly different in fed vs. fasted birds after 90 min in the water channel (Fig. 3A, repeated-measures ANOVA followed by Bonferroni's multiple comparison test). The temperature of the lower abdominal and the subcutaneous flank was the same at the start of the experiment, but the temperature decrease of the lower abdominal region was greater throughout the experiment (Fig. 3B).

Table 2. \dot{V}_{O_2} at rest (RMR_{air} and RMR_{water}) and during submaximal and maximal exercise for animals

	\dot{V}_{O_2} air, ml O_2 /min			\dot{V}_{O_2} water, ml O_2 /min		
	RMR_{air}	Submaximal Air	Maximal Air	RMR_{water}	Submaximal Water	Maximal Water
Fed	117.1 ± 20.0	246.6 ± 39.2	353.9 ± 80.3	114.8 ± 32.7	178.7 ± 38.9	301.6 ± 63.3
Fasted	86.9 ± 8.8	209.3 ± 41.2	283.7 ± 47.0	167.3 ± 36.7	237.7 ± 28.1	350.3 ± 32.9
	<i>P</i> values					
Fed vs. fasted	< 0.01	< 0.05	< 0.01	< 0.05	< 0.01	< 0.01
Fed (air vs. water)	> 0.8	< 0.05	> 0.1			
Fasted (air vs. water)	< 0.01	> 0.1	< 0.01			

Values are presented as means \pm SD; $n = 10$. RMR_{air} , resting metabolic rate in air; RMR_{water} , resting metabolic rate in water. *P* values are paired *t*-tests comparing mean values between fed vs. fasted, or between mean values in air vs. those in water, either fed or fasted.

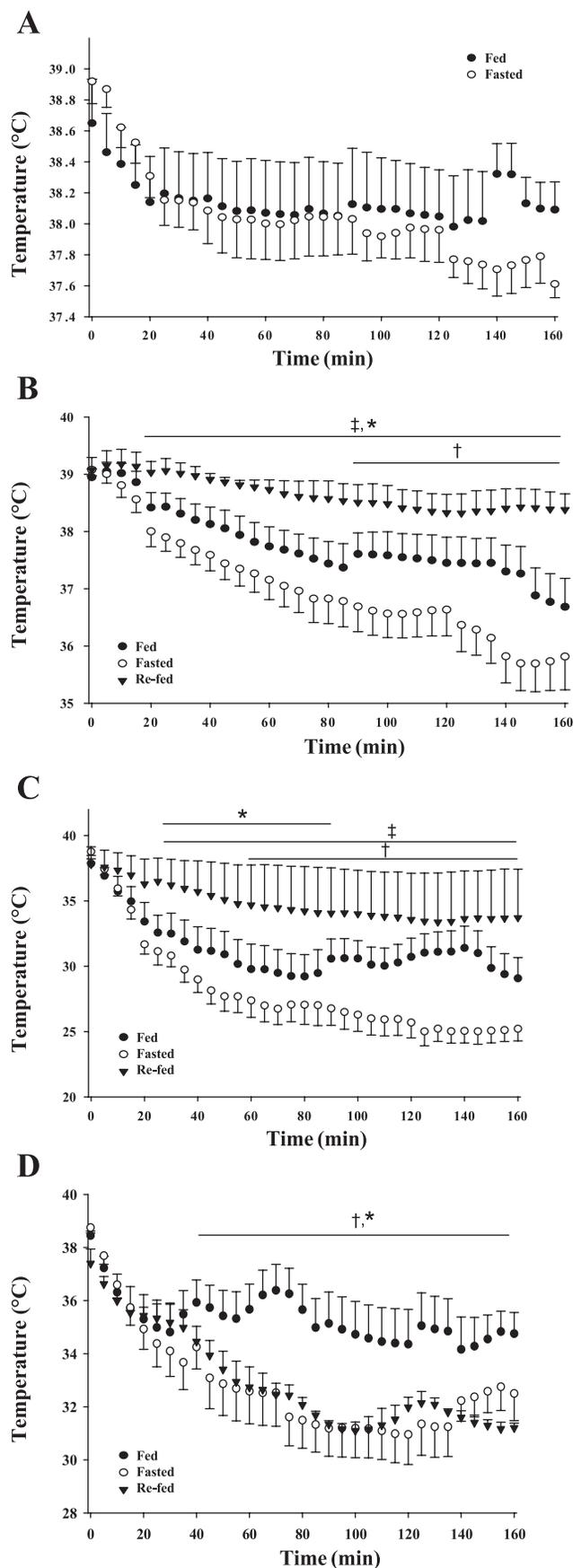


Table 3. Changes in body temperature while in water

	Upper Abdominal		Middle Abdominal		Lower Abdominal	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
Pre-Min	0.8±0.4	1.5±0.3	2.0±1.3	3.0±1.5	9.8±4.1	14.3±2.5
<i>P</i> value	< 0.01		< 0.01		< 0.05	
Pre-Mean	0.4±0.3	0.9±0.3	1.2±0.9	1.9±1.0	6.9±3.4	9.9±3.0
<i>P</i> value	< 0.05		< 0.01		< 0.01	

Values are expressed as means ± SD. Changes were computed as the difference in the body temperature (°C) immediately before (pre) minus the minimum (pre-Min) or mean body temperature (pre-Mean) during the experiment for the upper ($n = 7$), middle ($n = 9$), and lower abdominal ($n = 8$) temperatures before (fed) and after fasting (fasted). *P* values represent paired *t*-test fed and fasted.

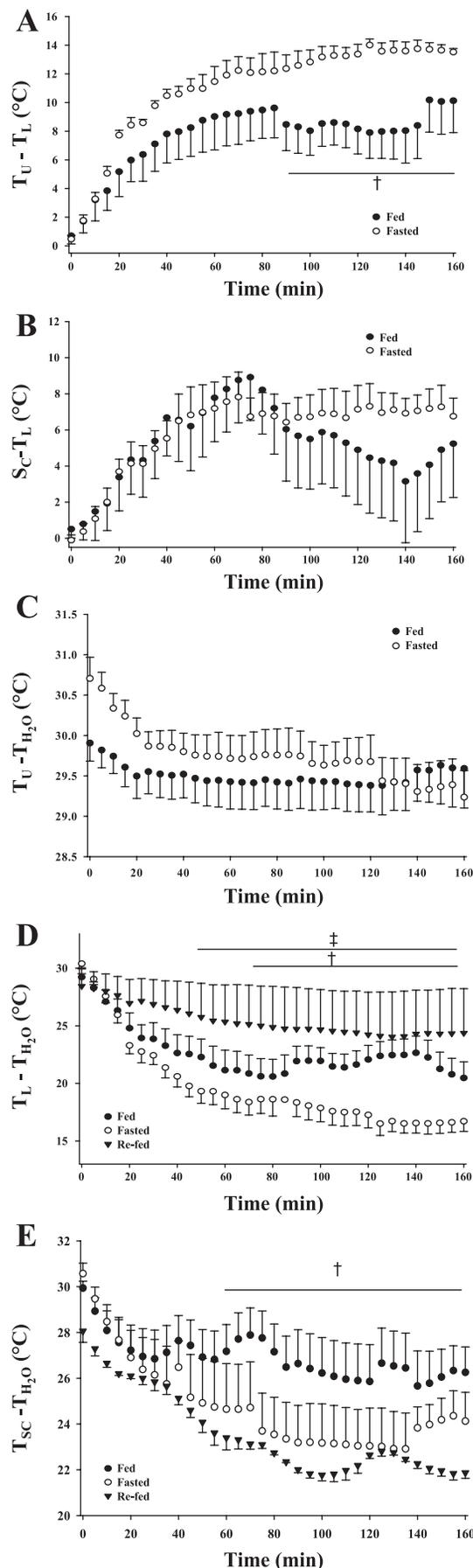
The differences between the lower abdominal and the water temperature and the subcutaneous and the water temperature were significantly different in fed vs. fasted animals after 60 min in the water channel (Fig. 3, *D* and *E*).

In the water channel, the thermal conductance changed during the first 30 min in the fed birds (Fig. 4). Following this, it remained more or less constant for the remainder of the experiment. In fasting birds, on the other hand, the thermal conduction remained more or less constant throughout the experiment. During the first 5 min in the water channel, the thermal conduction was the same before and after fasting, but after 30 min the thermal conduction was lower in fed animals (Fig. 4).

Refeeding

Comparing the same animals between the two successive fasting sessions, there was no difference in $\dot{V}O_2$ at rest, or during submaximal and maximal exercise in either water or air ($P > 0.2$, paired *t*-test). Therefore, the values from the end of these two fasting periods were averaged for each animal. Thus the fasting values reported for these four birds (Table 4) are the mean values from both fasting periods. The average $\dot{V}O_2$ for these four animals fasting and after refeeding are summarized in Table 4. Twenty four hours after refeeding in air, there was no difference in $\dot{V}O_{2\text{air}}$ at any exercise level compared with those in fasting birds. By contrast in the water channel, both submaximal and maximal $\dot{V}O_{2\text{water}}$ were lower in refeed than in fasting birds. It is important to note that when the birds were in water, $\dot{V}O_2$ during submaximal exercise was less in all four of the refeed individuals, whereas when in air, it was greater in 2 and less in 2, hence the difference in significance, despite the similar mean values and variances (see Table 4). There was also a trend for a 24% decrease in $\text{RMR}_{\text{water}}$ (Table 4). In the refeed group, temperatures from the middle, lower, and subcutaneous regions were only available for three birds. This explains their absence in Figs. 2A, 3, A–C, and 4. After

Fig. 2. Mean temperatures (mean ± SE) in various body compartments of king penguins immediately before (time = 0) and during 160 min throughout a water channel experiment, before (fed) and after fasting (fasted) and 24 h after a single refeeding event (refed). A: upper abdomen ($n = 7$ fed, $n = 7$ fasted, $n = 0$ refeed), B: middle abdomen ($n = 9$ fed, $n = 9$ fasted, $n = 3$ refeed), C: lower abdomen ($n = 8$ fed, $n = 8$ fasted, $n = 3$ refeed), D: subcutaneous fat ($n = 7$ fed, $n = 7$ fasted, $n = 3$ refeed). †Significant difference fed and fasted, ‡refed and fasted, or *refed and fed animals ($P < 0.05$, repeated-measures ANOVA followed by Bonferroni's multiple comparison test).



refeeding, the changes in subcutaneous temperatures were similar to those observed in fasting animals (Fig. 2D). In contrast, the initial temporal decrease in the middle (<1°C, Fig. 2B) and lower (<3°C, Fig. 2C) abdomen was significantly lower than both fed and fasted birds. As a consequence, the temperature difference between the lower abdomen and ambient water increased after refeeding (Fig. 3D).

DISCUSSION

In the current study, RMR_{water} in 8.5°C water after an average 14.5 days of fasting was 167.3 ml $O_2 \cdot \text{min}^{-1}$ and is similar to the mean RMR_{water} reported previously for fasted wild king penguins at 4°C (172.3 ml O_2/min) (17), or at 9°C (160 ml O_2/min) (12) at similar body mass. In addition, the current results showed an increase of 93% in RMR_{water} compared with RMR_{air} in fasting animals, and this corroborates earlier studies in king penguins (17) and in the little penguin, *Eudyptula minor* (40), where the differences in $\dot{V}O_2$ between air and water were between 74 and 144%. In the fed birds, that is, in penguins just returning from the sea, RMR_{water} was 114.8 ml O_2/min , which was not different from RMR_{air} (Table 2). This differs radically from previous research in most aquatic birds in which resting $\dot{V}O_2$ in cold water is usually two to three times as high as that in air (30, 40). However, in most other studies the nutritional status of the birds was not specifically taken into consideration, although RMR is often measured in animals that have been fasting for many hours (30, 40). Thus in animals that have been feeding regularly and have little or no body reserves, fasting for many hours could be physiologically similar to the fasting state of the birds in the present study. The latter were physiologically prepared for a relatively long fast by having relatively large body (fat) reserves. It would be interesting, therefore, to determine whether or not $\dot{V}O_2$ of nonfasting individuals of other species when in air and water are similar to each other, as they are in fed king penguins. Two possible explanations for the results obtained in the present study are given below.

Several past studies have attempted to “correct” oxygen consumption rates on a mass-specific basis ($\dot{V}O_2 \cdot M_b^{-1}$, ml $O_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$), even though there is no a priori reason to assume isometry (35). Interspecific allometric mass exponents for resting metabolic rate in air range from 0.66 to 0.92 (14, 38), but intraspecific mass exponents >1 have been reported in fasting birds (13, 16, 17, 27). Thus there is little reason to assume a direct relationship on a mass-specific basis within and between species. Therefore, without an appropriate analysis to confirm isometry, studies reporting mass-specific differences in metabolic rates are likely to convey erroneous conclusions (35). To avoid this problem, we previously derived the mass exponent for resting animals in air (1.89) (17), without making any a priori assumptions of what the correction factor should be.

Fig. 3. Temperature differences (mean \pm SE) between different regions of the body during water channel experiments before (fed) and after (fasted) fasting and 24 h after a single refeeding (refed) event in king penguins. Temperature differences are upper (T_U) – lower (T_L) abdominal ($n = 6$ fed, $n = 6$ fasted, $n = 0$ re-fed) (A); subcutaneous – T_L ($n = 6$ fed, $n = 6$ fasted, $n = 0$ re-fed) (B); T_U – water temperature (T_{H_2O} , $n = 7$ fed, $n = 7$ fasted, $n = 0$ re-fed) (C); T_L – T_{H_2O} ($n = 8$ fed, $n = 8$ fasted, $n = 3$ re-fed) (D); and T_{SC} – T_{H_2O} ($n = 7$ fed, $n = 7$ fasted, $n = 4$ re-fed) (E). †Significant difference fed and fasted ($P < 0.05$, paired t -test).

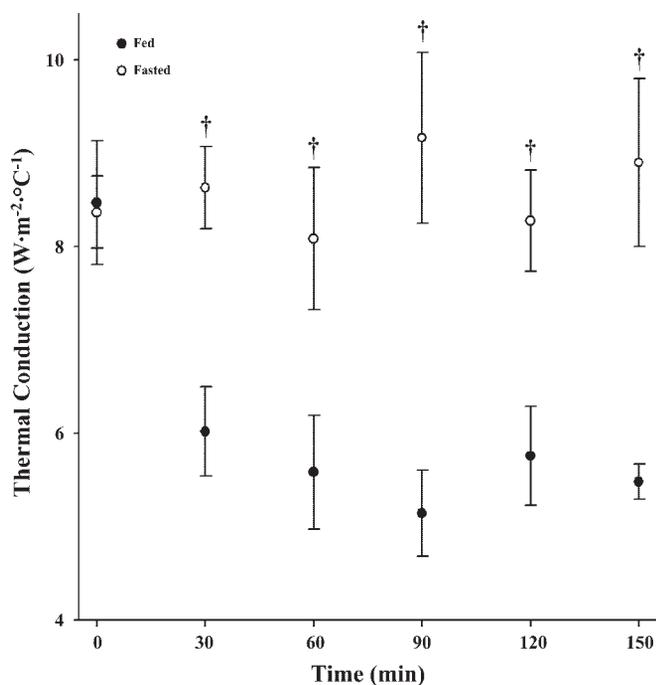


Fig. 4. Mean (\pm SE) thermal conductance ($\text{W}\cdot\text{m}^{-2}\cdot^{\circ}\text{C}^{-1}$) for upper abdominal temperature before (pre, $n = 7$), after (post, $n = 7$) fasting. †Significant difference between fed and fasted animals ($P < 0.05$, paired t -test).

We hypothesized that the large decrease in RMR_{air} during fasting in king penguins, an example of hypometabolism, was partly due to a decrease in body temperature. However, there was no change in temperature of the selected body core region, although we could not eliminate the possibility of a decrease of the volume of the body core. Nonetheless, the measurements of body temperatures from the current study argue against changes in body temperature as an explanation for the fasting-related hypometabolism. Considering the stable temperatures measured in air throughout fasting, a decrease in body core temperature is not an appropriate explanation of the apparent hypometabolic state observed in fasting king penguins in air. However, our results do not rule out other biochemical or molecular possibilities, including regulatory alterations of gene expression, changes in protein synthesis and degradation (42), or hormonal changes (11).

We further hypothesized that fasting would elicit a similar change in RMR of king penguins in water, or at least a limited decrease of $\dot{V}\text{O}_2$ as the thermal heat loss would increase with fasting. Contrary to this suggestion, and despite a 20% decrease in M_b , $\text{RMR}_{\text{water}}$ increased with fasting. In other words, $s\dot{V}\text{O}_2$ increased with fasting, and the resulting allometric mass

exponent was -1.45 . Thus fasting is associated with a decrease in mass-specific metabolism in air and an increase in water in the king penguin. This highlights the suggestion by Packard and Boardman (34, 35) that appropriate statistical tests and body mass corrections for metabolic rates are necessary in comparative studies.

In water, complex thermoregulatory changes suggest that there is a different explanation of these surprising results. Together with our previous results (17), they provide evidence of a complex interplay between fasting-related changes and physiological adjustments that allow maintenance of a more or less constant upper abdominal temperature, that is, the body core (Fig. 2A) with an associated concurrent regulation of $\dot{V}\text{O}_2$. On the other hand, subcutaneous flank (Fig. 2D), middle (Fig. 2B) and lower abdominal (Fig. 2C) temperatures vary over a much larger range in both fed and fasted animals.

The large negative allometric mass exponent with fasting could be related to the higher thermal conductance in fasting vs. fed birds in water ($2\text{--}3 \text{ W}\cdot\text{m}^{-2}\cdot^{\circ}\text{C}^{-1}$ higher than in fed birds, Fig. 4). The thermal conductance values observed in fasting animals are similar to those already reported in resting cold-adapted juvenile king penguins ($8.65 \text{ W}\cdot\text{m}^{-2}\cdot^{\circ}\text{C}^{-1}$) (1) of similar M_b (11.6 kg) (1) to the fasted adult birds in the current study (10.9 kg, Table 1). Thermal insulation in penguins is provided by the subcutaneous fat and the air trapped in the feathers (30). Assuming that the insulatory ability of the feathers is not affected by fasting, this high thermal conductance can be explained by a reduction in the subcutaneous fat insulation. In fasting penguins, the change in body fat during *phase II* is mainly due to the mobilization of subcutaneous depots, the major organ of body reserves in the king penguin ($\sim 47\%$ of total M_b) (10). A better insulation of the adipose tissue and an efficient vasoconstriction of the periphery when submerged in water allow fed birds to decrease their thermal conductance from 9 to $6 \text{ W}\cdot\text{m}^{-2}\cdot^{\circ}\text{C}^{-1}$ after 30 min inside the water channel (Fig. 4). This value is similar to that reported for nonbreeding, premolting Adelle penguins ($5.54 \text{ W}\cdot\text{m}^{-2}\cdot^{\circ}\text{C}^{-1}$) (30). This rapid increase in insulation [decrease in conductance (C), Fig. 4] observed in fed birds is presumably the main reason for the maintenance in water of $\text{RMR}_{\text{water}}$ identical to that measured in air (Table 2).

It is possible that the relatively higher activity level in the fasted birds (searching and swimming was 39.4% and 25.1% of the total activity in fasted and fed birds, respectively) could explain why fasted birds had a higher mean C, as increased activity would lead to increased metabolic rate and to increased convective heat loss. To analyze this, a mixed-model ANOVA of the form $C = a + b \times \text{fraction of activity}$, where fraction of activity was the fraction of observed activity for each 5-min

Table 4. $\dot{V}\text{O}_2$ at rest (RMR_{air} and $\text{RMR}_{\text{water}}$) submaximal and maximal exercise for animals in air or in water after fasting and 24 h after a single refeeding event (refed)

	$\dot{V}\text{O}_2$ air, ml O ₂ /min			$\dot{V}\text{O}_2$ water, ml O ₂ /min		
	RMR_{air}	Submaximal Air	Maximal Air	$\text{RMR}_{\text{water}}$	Submaximal Water	Maximal Water
Fasted	88.0 \pm 8.3	211.4 \pm 37.1	296.6 \pm 35.8	159.5 \pm 37.1	207.7 \pm 41.7	336.0 \pm 55.0
Refed	80.3 \pm 6.5	179.0 \pm 20.2	283.8 \pm 20.8	120.9 \pm 34.6	170.5 \pm 27.4	238.4 \pm 33.6
P values	> 0.3	> 0.2	> 0.8	< 0.1	< 0.05	< 0.01

Values are presented as means \pm SD; $n = 4$. The fasting values are mean values from two fasting experiments for the 4 birds. P values are paired t -tests comparing mean values between fasted versus refed.

period, and C the estimated thermal conduction for the same 5-min period was used to partition C in fed vs. fasted birds. We omitted the data for the first 30 min when $S \neq 0$. This analysis suggested that C in fed animals while swimming underwater was $7.6 \text{ W} \cdot \text{m}^{-2} \cdot ^\circ\text{C}^{-1}$, while the value in fasted animals was $9.6 \text{ W} \cdot \text{m}^{-2} \cdot ^\circ\text{C}^{-1}$. The comparable values for resting fed and fasted birds were 4.9 and $7.9 \text{ W} \cdot \text{m}^{-2} \cdot ^\circ\text{C}^{-1}$, respectively. In addition, there was no difference in the duration of underwater swimming in fasted vs. fed animals. Thus the higher C in fasted birds was most likely caused by changes in their physiology and/or morphology and not by changes in their behavior.

Even if the quality of subcutaneous insulation could explain part of the difference in mass specific $\dot{V}_{\text{O}_{2\text{water}}}$ observed between fed and fasting birds, the fact that the thermal conductance did not decrease in fasting birds in water argues for an attenuation of the peripheral vasoconstriction usually observed in aquatic endotherms in response to submergence in cold water (6). Furthermore, the trend for decreasing $\text{RMR}_{\text{water}}$ in refed birds (significant during activity, Table 4) implies an additional problem endured by fasting birds when submerged in water. We hypothesize that the metabolic and regional temperature changes in water with fasting are regulated to meet two conflicting demands. The first is to reduce thermal heat loss, and the other is the need to mobilize fuel from the subcutaneous adipose tissues during the fasting period ashore. The use of this major source of fuel requires a nominal level of blood perfusion, that is, vasodilatation, which, in turn, increases peripheral heat loss. In this context, maintaining constant temperature of the body core would become impossible without increasing $\dot{V}_{\text{O}_{2\text{water}}}$.

Twenty four hours after a single refeeding event, the middle and abdominal temperatures increased compared with the fasted animals (Fig. 2, *B* and *C*). However, despite the apparent reperfusion of the middle and lower abdominal regions, the reduction in $\dot{V}_{\text{O}_{2\text{water}}}$ suggests reduced overall heat loss (Table 4). Increased perfusion to the gut allows extraction of nutrients and restoration of the abdominal fat pad (Fig. 1, *B* and *C*). Extraction of nutrients from the gut to the blood occurs mainly by passive diffusion. However, there is active uptake of nutrients such as monosaccharides, amino acids, and B-complex vitamins (41), but as RMR_{air} was not different before and 24 h after refeeding (Table 4), this active uptake does not appear to add much to the overall metabolic rate of the animal. In addition, restoration of the abdominal fat pad increases the insulation of the lower abdominal region. This agrees with the general interpretation in other animal models in which the abdominal fat is the first resource to become exhausted during fasting and the first restored during refeeding while subcutaneous tissues, in contrast, are the last to become restored during refeeding (2, 4, 25).

The fact that the decrease in the temperature of the lower abdominal tissue was greater than that of the subcutaneous flank is an apparent paradox (Fig. 3*B*). That is, the heat loss from a more central tissue, the lower abdomen, was higher than from a more peripheral tissue, the subcutaneous flank. This argues for a highly complex and partitioned blood perfusion of the more marginal tissues of the body core (the lower abdomen). One possibility is a fasting-related adjustment of the thermal conductance (between body core and ambient water; Fig. 4) resulting from local vasoconstriction of different abdominal regions in alternating sequences and of different areas

of the skin. This would create insulatory barriers that reduce heat loss from the thermal core or alternatively, create local avenues for increased heat loss from the lower abdominal region. The brood patch could play a particular role in the adjustment of heat loss from the lower abdomen and may be controlled independently of the general blood perfusion of the feathered part of the skin.

The current results are similar to those previously reported for animals at sea, where a large temperature difference could exist between the upper and the lower abdomen ($>10^\circ\text{C}$) (26), even though the tissues are less than 5 cm apart. Consequently, an active and rapid decrease of the temperature of the lower abdomen, especially in fasted birds for which lower abdominal activity is not necessary, could reduce the increased \dot{V}_{O_2} when transferred from air to water both by a Q_{10} effect and by reducing the thermal gradient. There was a more rapid and extreme decrease in the temperature of the lower abdomen in fasted compared with fed birds (Fig. 2*C*). This could be a compensatory mechanism in fasted birds to reduce local heat production linked with their incapacity to reduce their overall thermal conductance, while maintaining a stable core temperature. This agrees with data from freely diving and foraging birds, where a complex interplay between the deep core and brood patch temperatures is suggested to enhance the bird's ability to remain submerged (Schmidt A, Alard F, and Handrich Y, unpublished observation). In birds given a single meal after a period of fasting, the temperature of the subcutaneous flank remained low, and this is suggestive of a complete vasoconstriction of the feathered parts of the skin and possibly also of the brood patch. The temperatures of the lower and middle abdomen (Fig. 2*B* and *C*), on the other hand, indicate vasodilatation, in contrast to the situation in fully fed and fasted animals. This suggests that the refed animals perfused the splanchnic region to access nutrients in the gut and to restore the fat depot in this region.

Barré and Roussel (1) concluded that the physiological adaptations to a semiaquatic life in juvenile penguins involved an internal insulatory reinforcement, possibly due to an improvement in the ability to vasoconstrict the periphery, but also due to an increase in the thermogenic capacity. One suggestion was that the juvenile birds were unable to vasoconstrict as well as the adult. However, the present study provides an alternative explanation and suggests a trade-off between thermoregulation and the access to peripheral fat depots during the return to the sea after an extended fast.

In conclusion, few studies have investigated the physiological responses of penguins during submergence in water and in air. If we are better to understand the metabolic requirements of these animals during their annual cycle, more studies are required to appreciate the physiological plasticity of these animals. The present data emphasize the problem with reporting metabolic rate on a mass-specific basis, and may reveal some new complex features of the thermoregulatory physiology of king penguins. The data suggest that the changes in metabolic rate and regional temperature in water with fasting and refeeding can be explained by: 1) the level of subcutaneous insulation, 2) the need to protect the body core from extreme changes in temperature and 3) the need to mobilize body fuel from the subcutaneous adipose tissues during the fasting period ashore.

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