

RENAL FUNCTION AND PLASMA LEVELS OF ARGININE VASOTOCIN DURING FREE FLIGHT IN PIGEONS

ITAMAR GILADI¹, DAVID L. GOLDSTEIN², BERRY PINSHOW^{1,*} AND RÜDIGER GERSTBERGER³

¹Jacob Blaustein Institute for Desert Research and Department of Life Sciences, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, 84990 Israel, ²Department of Biological Sciences, Wright State University, Dayton, OH 45435, USA and ³Max-Planck-Institut, L. Physiologische U. Klinische Forschung, W. G. Kerckhoff-Institut, Parkstrasse 1, D-61231 Bad Nauheim, Germany

Accepted 19 September 1997

Summary

We examined urinary water loss and plasma levels of arginine vasotocin (AVT) in free-flying, tippler pigeons trained to fly continuously for up to 5 h. First, we used [³H]polyethyleneglycol ([³H]PEG) as a glomerular filtration marker by implanting an osmotic minipump into each bird. In two flights (10 birds in winter at an ambient temperature of 13–15 °C and seven in summer at 23 °C), we measured pre-flight (hydrated, resting control birds) and post-flight [³H]PEG activity and osmolality in blood and ureteral urine. For comparison, we measured these variables in 10 birds in winter before and after controlled dehydration (24 h at 25 or 30 °C). Second, we measured plasma levels of AVT in 6–8 birds before and immediately after each of three different summer flights. Urine osmolality increased significantly by up to three times the control level in both post-flight and dehydrated pigeons; urine:plasma osmolality ratios did not exceed 2. Compared with controls, glomerular filtration rate (GFR) was

significantly lower after flight in summer, but did not change in either post-flight or dehydrated winter pigeons. In winter, mean post-flight urine flow rate (UFR) decreased significantly to less than half the control level, while in summer, post-flight UFR did not differ from control levels. In general, mean filtered water reabsorption (Fr_{H_2O}) increased from 95 % in controls to 98 % in post-flight and dehydrated birds. Plasma levels of AVT increased after flight to between three and eight times the preflight levels. The data from this first study of kidney function during flight are consistent with previous studies of dehydration in birds and exercise in mammals in which both increased Fr_{H_2O} and decreased GFR contribute to renal conservation of water.

Key words: avian osmoregulation, kidney function, free flight, water balance, pigeon, tippler pigeon, *Columba livia*, arginine vasotocin, AVT, avian anti-diuretic hormone, dehydration.

Introduction

Bird flight represents one of the most impressive examples of sustained exercise among animals. During flight, birds expend energy at rates 10–15 times their resting levels (e.g. Berger *et al.* 1970; Rayner, 1982; Bernstein, 1987; Butler and Woakes, 1990). Such high levels of energy expenditure require concomitant increases in ventilation to supply adequate respiratory gas exchange, and the enhanced ventilation inevitably results in increased rates of evaporative water loss from the respiratory system. Birds also have significant rates of evaporation through their skin (e.g. Marder and Ben-Asher, 1983; Webster and King, 1987; Webster and Bernstein, 1987), and high rates of convection during flight could thus lead to increased cutaneous evaporation as well. Hence, total evaporative water loss may be substantially elevated during flight compared with rest (Adams *et al.* 1997; Giladi, 1997).

Birds could counter some of these elevated evaporative losses by minimizing the excretion of water. The avian kidney

produces urine by the processes common to all vertebrates – namely, initial filtration of the plasma in the glomeruli, followed by reabsorption of water and many solutes in the renal tubules (e.g. Sturkie, 1986). In birds, urine can be further modified in the cloaca and the lower parts of the gastrointestinal tract (e.g. Anderson and Braun, 1985; Goldstein and Braun, 1986). Conservation of urinary water could therefore be effected either by a reduced glomerular filtration rate (GFR) and/or by enhanced rates of water reabsorption. In humans (the mammals for which many data are available), the effects of exercise on kidney function include a reduction in renal blood flow rate and, particularly at heavier work loads, a reduction in GFR by up to 40% (Poortmans, 1994). Moreover, urine flow rate is even more reduced, by as much as 75% (Zambraski, 1990; Poortmans and Vanderstraeten, 1994), indicating that tubular reabsorption of water is also enhanced. In contrast, GFR and urine flow rate

*Author for correspondence (e-mail: pinshow@bgumail.bgu.ac.il).

may be elevated during exercise in other mammals (Grignolo *et al.* 1982). In birds, both reduced GFR and enhanced tubular water reabsorption typically occur during dehydration (e.g. Roberts and Dantzer, 1989; Williams *et al.* 1991). However, these processes have never been studied in exercising birds.

If excretory water loss is minimized during flight, it is likely that this occurs, in part, through the actions of the avian antidiuretic hormone arginine vasotocin (AVT). The increased release of AVT in dehydration is well documented (e.g. Stallone and Braun, 1986a; Gray and Erasmus, 1988b), and the consequences of this hormone's activity include a decreased GFR (Braun and Dantzer, 1974; Stallone and Braun, 1985; Gray and Erasmus, 1988a) and an increased renal tubule water reabsorption (e.g. Stallone and Braun, 1986b), both of which lessen urine flow rate and thus save water. However, the contribution of AVT to water conservation in exercising birds is unexplored.

In order to accommodate the functions of the vascular system during prolonged flight, birds must maintain blood volume (Carmi *et al.* 1993). Thus, the premises that (a) GFR decreases during dehydration in birds, (b) urine output is reduced (in mammals) during heavy exercise and (c) plasma AVT concentration increases during dehydration in birds led us to hypothesize that birds minimize excretory water loss during long flights and that this is accomplished in part through the actions of AVT. We investigated this hypothesis by testing the following predictions: (1) that water restriction induces renal conservation of water in tipler pigeons as in other birds; (2) that GFR is reduced during free flight compared with rest; (3) that renal water reabsorption is enhanced during flight; and (4) that the plasma concentration of AVT increases during flight.

Materials and methods

Experimental animals

Trained tipler pigeons were used in experiments that took place at the Jacob Blaustein Institute for Desert Research in the central Negev Highlands (34°47' N, 30°52' E), Israel. Tipler pigeons are a breed of rock pigeon (*Columba livia* Gmelin) that has been selected for their propensity to fly continuously for hours in large circles above their cote. The cote was an outdoor aviary with a sun- and wind-protected area, but otherwise exposed to ambient weather conditions. Our training protocol involved simultaneous release of the birds from the cote 3–5 times a week for training flights of 2–5 h. Birds aloft remained in view of their trainer for the duration of the flight and were conditioned to return to their cote at the sound of a whistle. Food was presented while the whistle was blown and then removed after 20 min. On training days, birds were fed immediately upon their return to the cote at the end of a flight. On non-flight days, birds were fed once a day for 20 min; water was available *ad libitum*. For experimental flights, water was unavailable prior to flight, and after landing access to water and food was denied until the sampling procedure was complete. Experimental birds were flown along with a number

of non-experimental individuals, so that a flock of 12–14 birds was released together. There were no apparent differences between the flight performance of experimental and of non-experimental birds.

Physiological measurements

Kidney function

We measured renal function using the approach first applied by Jobin and Bonjour (1985) and subsequently adapted for birds by Goldstein and Braun (1988). The principle behind this approach is that implanted osmotic minipumps infuse a filtration marker (e.g. polyethylene glycol, PEG) into the animal at a steady rate. The marker is then lost from the extracellular fluid solely by renal filtration. Because of this, the steady-state concentration of the marker in the plasma (extracellular fluid) is determined by the glomerular filtration rate (GFR). Once filtered, urinary marker concentration is then determined by the rate of water reabsorption by the renal tubules. Knowledge of the infusion rate and the plasma and urine concentrations of the marker therefore allows calculation of the GFR, the rate of tubular water reabsorption and, consequently, the urine flow rate.

The pumps (Alza, Inc., model 2001 or 1007D) were implanted into the peritoneal cavity under local (Lidocaine) anesthesia through a small incision in the body wall between the posterior end of the sternum and the ischium. Each pump contained approximately 100 μCi (3.67 MBq) of [^3H]PEG (New England Nuclear), sufficient in each case to provide approximately 20–60 $\text{disintegrations min}^{-1} \text{ml}^{-1}$ plasma. The incision was closed with two or three sutures, and birds were given 1.5–2 days to recover before experimental use.

Blood and urine samples were collected from all birds just before the start of an experimental manipulation (flight or dehydration) and again at the end. Birds were weighed to the nearest 0.1 g (Ohaus, model CT1200-S). Blood was obtained in all cases by puncture of a tarsal vein, so as not to affect flight performance adversely. Ureteral urine was sampled following Thomas *et al.* (1984) by inserting a cannula made from a 4 ml polyethylene centrifuge tube into the cloaca. A hole in the side of the cannula was placed dorsally, opposite the ureteral papillae, to collect urine draining from the ureters; the closed end of the cannula prevented contamination of urine by intestinal fluids. Typically, urine flow was relatively copious prior to flight, but the birds produced relatively little urine after flight; we collected approximately 50–100 μl of urine before flight but only 20 μl subsequent to it.

To obtain post-flight samples, which we took as indicative of physiological condition during flight, birds were captured and placed in dark holding bags immediately upon landing at their cote. Post-flight blood and urine samples and body masses were then taken as rapidly as possible; sampling of all birds was completed within 30 min (less than this for blood, which was sampled before urine). We saw no effect of sampling sequence (i.e. of time between landing and sampling) on the values of any variables that we measured (analysis of variance, all $P > 0.1$ for variables *versus* sampling interval).

To test whether fractionation of ^3H occurred between [^3H]PEG and the water in which it was dissolved, we incubated solutions of [^3H]PEG for up to 65 h at 40 °C, recovered the water by distillation and ran the distillate in the scintillation counter as described below. In no case were counts above background levels. Thus, we conclude that no measurable error was induced by ^3H fractionation in our experiments.

Effect of flight on plasma AVT levels

Blood was sampled and body mass measured just before and immediately after flight (before and after rest for the control birds). We collected 0.4–0.6 ml of blood in heparinized capillary tubes by puncture of the tarsal vein. The blood was immediately transferred to a cooled 4 ml, capped centrifuge tube containing 25–30 ml of 0.125 mol l⁻¹ sodium–EDTA and was kept on ice. Within 20 min, blood was centrifuged (2500 g) for 10 min at 4 °C, and the plasma was kept frozen (–20 °C) until assay.

General protocol

First, we examined the effects of flight and mild dehydration on kidney function. To achieve this, we implanted osmotic minipumps into the peritoneal cavity of each of seven pigeons in late summer (September), and 10 pigeons in winter (December). During the following 6 days, in the winter experiment, each bird underwent one test for the effects of mild dehydration and two experimental flights (five birds were dehydrated on days 2–3 after surgery and flew on days 5 and 7, the other five were dehydrated on days 3–4 and flew on days 2 and 6). In summer, we did not repeat the test for dehydration, but again flew each bird twice (on days 3 and 5 after pump implantation). In both winter and summer, the data for the two flights per individual did not differ, and so (to avoid statistical pitfalls) we arbitrarily chose one of the two flight days in each season (the same day for all birds) for analysis.

To examine the effects of mild dehydration on kidney function, each bird was removed from its cote and placed in an individual cage, without food and water, in a temperature-controlled room for 24 h. We tested the first group at 25 °C. To enhance dehydration in the second group, we increased the temperature from 25 to 30 °C for the second half of the test period.

While the birds were flying, we measured dry- and wet-bulb temperatures every 30 min using a sling psychrometer (Bacharach, 12-7013) on a 9 m tower under the birds' flight path. Ambient temperature (T_a) during the winter flights, which lasted 4 and 4.3 h (beginning at 07:30 and 08:00 h), varied between 10 and 17 °C. In summer, flight T_a was between 21 and 25 °C, and both flights were 3.8 h long (beginning at 05:45 and 06:30 h).

We examined the effects of flight on plasma concentrations of AVT on three different summer days. These experiments were carried out with the same stock of pigeons that was used for the kidney function experiments and under the same training regimen. In each experiment, we sampled a group of flying birds and a second group of resting control birds which

were kept in the cote under outdoor conditions without access to water or food for the duration of the flight. To control for the effect of individual, we interchanged flight and control groups for the third experiment. Flight duration of both AVT experiments was approximately 3.5 h, with mean ambient temperatures of 24.3 and 26.2 °C, respectively.

Sample and data analyses

Blood samples (other than for AVT analysis) were centrifuged for 5 min at 12 000 g to separate the cells from the plasma, the hematocrit was recorded, and the plasma was saved for analysis. Urine was similarly centrifuged, and the supernatant was analyzed. Samples were refrigerated pending analysis, which was always completed on the day of sampling. Osmolality was measured using a vapor pressure osmometer (Wescor, 5100C). Radioactivity was assayed by liquid scintillation counting (Packard Tri-Carb, model 1600TR), using 25 μl of plasma or 5–20 μl of urine (depending on the volume available) in 3 ml of scintillation fluid (Lumasafe). All measurements were made in duplicate unless sample size was inadequate.

The GFR, urine flow rate (UFR) and fractional reabsorption of water by the renal tubules were calculated following Goldstein and Rothschild (1993) and Goldstein (1995). Calculations similar to those of Goldstein and Rothschild (1993) showed that, on the one hand, the duration for which our pigeons flew was adequate to allow the plasma concentration of PEG to re-equilibrate if GFR changed during flight and, on the other hand, that the time between landing and post-flight sampling was sufficiently brief to ensure little change in plasma concentration of PEG. Thus, values of GFR measured just after landing should be representative of those during flight.

AVT samples were extracted with chilled acetone according to Gray and Simon (1983) and subjected to specific radioimmunoassay using monoradioiodinated AVT as the ligand. Intra- and inter-assay variability were 7.5 and 8.0 %, respectively, and recovery amounted to 92 %.

We tested for the effects of flight or dehydration on kidney function and AVT level using paired *t*-tests. Differences between seasons or pre-flight conditions in different flights were analyzed using one-way analysis of variance (ANOVA). We used Sigmastat (Jandel Scientific) for statistical analysis of the data and for calculating the power of each parametric test that we used. Where the assumptions of the parametric tests were not satisfied, or the power of the test was not sufficient ($\beta > 0.2$), we used the new, more robust non-parametric resampling statistics (Simon, 1995) to carry out the non-parametric analogs of the paired *t*-test and the permutation test (Siegel and Castellan, 1988). Data are presented as means ± 1 s.e.m. Differences were considered significant if $P \leq 0.05$.

Results

Pre-flight conditions

Combined, all the pigeons in which we measured kidney function had a mean pre-flight body mass (m_b) of 283 \pm 5 g (data

from Table 1); mean m_b in these birds did not differ significantly between the winter and the summer groups. Masses of birds at rest in which we measured AVT levels were similar (Table 2). Pre-flight hematocrit did not differ among all summer experiments, with means ranging from 50.1 ± 1.5 to $52.7 \pm 0.8\%$ (Tables 1, 2), but in winter it was significantly higher ($55.6 \pm 0.8\%$) than in summer (Table 1). In contrast, plasma osmolality did not differ between seasons, and averaged $320\text{--}330$ mosmol kg^{-1} in the various groups of resting birds (Tables 1, 2).

GFR before flight averaged approximately 65 ml h^{-1} in summer and was significantly lower (40 ml h^{-1}) in winter. Of this filtered load, 96% (not significantly different in winter *versus* summer) was reabsorbed in the renal tubules, resulting in a urine flow rate of 2.0 ml h^{-1} (summer) and 1.7 ml h^{-1} (winter). Pre-flight urine osmolality was significantly higher in summer (465 mosmol kg^{-1}) than in winter (120 mosmol kg^{-1}) (Table 1). Resting plasma concentration of AVT averaged 12.4 ± 1.8 to 20.0 ± 3.3 pg ml^{-1} and did not differ significantly between different flights or between flight and control groups (Table 2) (Kruskal–Wallis one-way ANOVA on ranks, d.f.=5, $P=0.1$).

Effects of dehydration on kidney function

The dehydration that we imposed produced a moderate response. The birds lost an average of 31 g, approximately 10% of m_b . However, some (unquantified, but probably substantial) fraction of this loss was dry excreta, not net loss of body water, and neither hematocrit nor plasma osmolality changed significantly during water restriction (Table 1). Similarly, GFR did not differ before and after dehydration. Urine flow was reduced, however, apparently because of elevated fractional reabsorption of water. Urine osmolality was significantly higher in the birds after dehydration.

Effects of flight on kidney function

All birds lost body mass during flight. In summer, mass loss

averaged 31.7 ± 8.6 g, or approximately 11% of m_b . In contrast, mass loss in winter was significantly lower, 15.1 ± 5.0 g, or 5.4% of m_b ($t_{10}=4$, $P<0.01$). In both summer and winter, hematocrit was lower and plasma osmolality higher in birds after flight than before. The changes were significantly different between seasons (hematocrit, $t_{14}=3.2$, $P<0.01$; plasma osmolality, $t_{14}=6.3$, $P<0.001$); however, the change in plasma osmolality was greater in summer, and the change in hematocrit was greater in winter (Fig. 1).

Winter pre- and post-flight GFR values did not differ. Fractional reabsorption of water tended to increase, although the difference was not statistically significant ($P=0.06$). Similarly, although not statistically significant ($P=0.07$), there was a trend towards reduced urine flow rate. The significant increase in urine osmolality strongly suggests that these trends are real.

In summer, GFR decreased significantly during flight (Table 1). Again, we could not detect a significant decrease in urine flow rate or a significant increase in fractional water reabsorption even though the apparent changes were of the same order of magnitude as those of winter birds (Table 1). We suspect that in both winter and summer this might be due to the high variation in both urine flow rate and fractional water reabsorption prior to flight (but see Discussion). We found no significant change in urine osmolality from the already high and variable values in pre-flight summer birds.

Effects of flight on plasma AVT levels

The range of mass loss during flight in birds used to measure AVT concentrations was 27.5 ± 3.2 to 37.7 ± 3.0 g (9–13% of m_b) (Table 2) and was similar to that of birds flown in summer for measurement of kidney function (Table 1). Plasma osmolality increased and hematocrit decreased significantly in all flights, whereas neither variable changed in any consistent direction in the resting, control birds. A significant increase in plasma AVT levels at rest was found only in the pigeons that

Table 1. Variables associated with kidney function during moderate dehydration and free flight in tipler pigeons

	Winter			Summer	
	Pre-flight	Post-flight	Dehydration†	Pre-flight	Post-flight
Body mass (g)	281.0 ± 4.3 (10)	265.9 ± 4.4 (10)**	274.6 ± 5.0 (10)*	285.0 ± 10.5 (6)	253.4 ± 7.6 (6)**
Hematocrit (%)	55.6 ± 0.8 (10)	51.5 ± 0.9 (10)**	54.6 ± 1.3 (10)	50.4 ± 1.2 (6) ^a	48.2 ± 0.8 (6)*
P_{osm} (mosmol kg^{-1})	319.9 ± 1.4 (10)	328.3 ± 2.5 (10)**	324.4 ± 2.0 (10)	321.2 ± 1.3 (6)	355.9 ± 4.7 (6)**
GFR (ml h^{-1})	39.7 ± 2.1 (10)	41.5 ± 2.7 (9)	38.7 ± 1.8 (10)	65.1 ± 3.6 (6) ^b	52.7 ± 4.1 (6)*
$\text{Fr}_{\text{H}_2\text{O}}$ (%)	95.4 ± 1.1 (10)	98.2 ± 0.2 (9)	98.6 ± 0.3 (9)*	96.8 ± 1.9 (6)	98.5 ± 0.1 (5)
U_{osm} (mosmol kg^{-1})	120.0 ± 6.3 (7)	689.3 ± 65.5 (6)**	535.8 ± 48.9 (4)**	465.2 ± 101.8 (4) ^a	548.8 ± 38.1 (4)
UFR (ml h^{-1})	1.7 ± 0.4 (10)	0.74 ± 0.1 (9)	0.56 ± 0.12 (9)*	2.0 ± 1.1 (6)	0.7 ± 0.05 (5)

Birds were flown in winter or summer. In winter, they were also subjected to 24 h of moderate dehydration at $25\text{--}30$ °C.

P_{osm} , plasma osmolality; U_{osm} , urine osmolality; GFR, glomerular filtration rate; UFR, urine flow rate; $\text{Fr}_{\text{H}_2\text{O}}$, fractional water reabsorption (proportion of the water that is filtered in the kidney that is reabsorbed in the kidney).

Values are means \pm s.e.m. The sample size is given in parentheses.

For pre- *versus* post-flight intra-season comparisons, * $P<0.05$, ** $P<0.01$; for inter-season comparisons, ^a $P<0.05$, ^b $P<0.01$.

†All data for dehydrated birds except body mass were compared with pre-flight resting values. Post-dehydration body mass was compared with pre-dehydration resting body mass, 305.1 ± 6.4 g ($N=10$).

Table 2. Comparisons of body mass, hematocrit, plasma osmolality (P_{osm}) and arginine vasotocin (AVT) levels in tipler pigeons before and after approximately 3.5 h of free flight with those of control birds that rested in their loft at the same time and under the same environmental conditions

Rest	24.3 °C		24.7 °C		26.2 °C	
	Pre-rest	Post-rest	Pre-rest	Post-rest	Pre-rest	Post-rest
Body mass (g)	281.2±8.4 (8)	265.9±8.8 (8)**	278.1±10.0 (7)	268.2±10.3 (7)**	284.6±8.8 (7)	274.9±8.3 (7)**
Hematocrit (%)	50.1±1.53 (7)	45.3±1.3 (4)	51.07±1.3 (7)	49.6±1.2 (7)	51.5±0.7 (7)	51.9±0.6 (7)
P_{osm} (mosmol kg ⁻¹)	327.5±3 (8)	319.3±2.55 (6)*	319.1±1.7 (7)	328.6±2.55 (7)**	325.0±1.1 (5)	327.1±1.1 (6)
AVT (pg ml ⁻¹)	15.4±1.04 (8)	18.34±3.7 (7)	14.5±4.2 (6)	22.1±5.6 (6)	17.7±0.8 (7)	29.5±3.2 (7)*

Flight	24.3 °C		24.7 °C		26.2 °C	
	Pre-flight	Post-flight	Pre-flight	Post-flight	Pre-flight	Post-flight
Body mass (g)	297.7±9.2 (8)	270.3±9.1 (8)**	296.3±11.2 (7)	263.2±9.3 (7)**	294.9±9.9 (6)	257.1±7.5 (6)**
Hematocrit (%)	52.5±1 (8)	48.7±0.8 (8)*	52.7±0.8 (6)	51.0±0.6 (7)*	51.0±1.6 (6)	47.6±1.6 (5)*
P_{osm} (mosmol kg ⁻¹)	319.1±2.5 (8)	345.6±7.3 (8)*	320.0±2.0 (7)	353.4±5.1 (7)**	321±2.0 (6)	359.9±1.6 (6)**
AVT (pg ml ⁻¹)	20.0±3.3 (8)	73.4±5.1 (7)**	16.9±2.3 (7)	87.9±6.9 (7)**	12.4±1.8 (6)	95.4±8.1 (6)**

Values are presented as mean ± S.E.M.; sample size is given in parentheses.

For pre- versus post-treatment comparisons, * $P < 0.05$, ** $P < 0.001$.

Birds were flown on three different days, at different temperatures (see text for further details).

served as controls for the flight at the highest ambient temperature in our study (Table 2).

The effect of flight on plasma AVT levels was very strong; post-flight levels were 3–8 times pre-flight levels. The difference was significant in all flights (Table 2). In order to test for the effects of changes in body mass, post-flight plasma osmolality and post-flight hematocrit on post-flight AVT concentration, we combined data from all flights, each bird being represented only once (for birds for which more than one value was available, we randomly chose one). Body mass loss

(x , in % initial m_b) was significantly correlated with post-flight plasma AVT concentration (y in pg ml⁻¹), where $y = -12.4 + 8.4x$ ($N = 13$, $r^2 = 0.37$, $P = 0.03$), but neither plasma osmolality nor hematocrit was significantly correlated with post-flight plasma AVT concentration ($N = 12$, $r^2 < 0.01$, $P > 0.7$ for both).

Discussion

Kidney function and plasma levels of AVT at rest

The GFR predicted for a normal hydrated bird with the mass of the pigeons we studied is 55–60 ml h⁻¹ (Roberts *et al.* 1985; Williams *et al.* 1991). The mean GFRs that we measured were close to this range in summer (65 and 53 ml h⁻¹ pre- and post-flight, respectively) but lower in winter (40 ml h⁻¹ for all treatments). The reason for this significant difference in GFR is not clear. All birds were exposed to ambient environmental conditions, and heat acclimation of pigeons does lead to changes in other characteristics of water balance such as rates of cutaneous evaporation (Marder and Arieli, 1988); however, we are unaware of data concerning seasonal GFR changes in birds. The difference in GFR was also associated with a significant difference in hematocrit (lower in summer), possibly indicative of a relative expansion of the extracellular fluid (plasma) volume in summer. Higher hematocrit in winter compared with summer is known from other species of birds (e.g. Clemens, 1990; Swanson, 1990). Nevertheless, despite the higher GFR and lower hematocrit, pigeons in summer also had higher urine osmolalities.

AVT levels in plasma of resting birds in the present study were 12.4–20 pg ml⁻¹. These levels are similar to the 16 pg ml⁻¹ found previously for resting homing pigeons (George *et al.* 1992), although somewhat higher than those reported in some other species of birds (Stallone and Braun, 1986a,b; Gray and Erasmus, 1988b; Jungbluth *et al.* 1994).

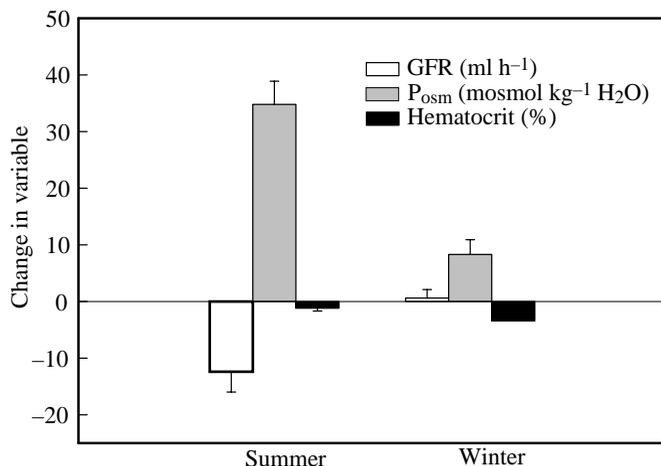


Fig. 1. Comparison of changes in glomerular filtration rate (GFR), plasma osmolality (P_{osm}) and hematocrit between tipler pigeons before (resting, control) and after 3–5 h of free flight in summer and winter. Changes are in absolute values of each variable: GFR in ml h⁻¹, P_{osm} in mosmol kg⁻¹, hematocrit in %. Values are means ± 1 S.E.M. Sample size for summer, 10; for winter, 6. The change in P_{osm} was significantly greater in summer than in winter, whereas the change in hematocrit was significantly greater in winter. For further details, see text.

Effects of dehydration

The typical response of avian renal function to dehydration is a reduction in urine flow effected by a diminished GFR and elevated tubular water reabsorption (e.g. Goldstein and Braun, 1988; Williams *et al.* 1991). The presumed mediator of these actions is AVT. In general, AVT elicits both a tubular response (enhanced water reabsorption) and a reduction in GFR, although at low doses its effect on GFR differs among species (e.g. Stallone and Braun, 1985; Gray and Erasmus, 1988*b*; Gerstberger *et al.* 1985).

In our pigeons, we imposed a relatively modest dehydration challenge (24 h at 25–30 °C); even dehydration for 36 h at 25 °C in birds of the same stock (Carmi *et al.* 1993) resulted in little change in plasma osmolality and no change in plasma volume (the primary stimuli for the release of AVT). Thus, it is likely that the dehydration regimen used in the present study would have resulted in only a small elevation of AVT concentration similar to that found for birds resting at 26.2 °C in the AVT experiments. The observation that mildly dehydrated pigeons reduce their urine flow rate by changing fractional water reabsorption, without any change in GFR (Table 1), is then consistent with the actions of AVT in the domestic fowl (Stallone and Braun, 1985).

*Effects of flight**Mass loss, plasma osmolality and hematocrit*

We predicted that flight would induce similar changes to dehydration; that is, that pigeons would conserve water by minimizing renal excretion of urine. Indeed, the stimuli imposed by flight in our experiments included a significant loss of body mass, virtually all of which represents loss of body water (Giladi, 1997); this apparently induced a rise in plasma osmolality.

Despite the substantial loss of body water, hematocrit in flying pigeons decreased in both winter and summer. In previous studies of thermal dehydration, pigeons have been shown to retain vascular fluid volume even in the face of marked loss of body water (Carmi *et al.* 1994). In both situations, this probably results primarily from a shift of water into the vascular system from the interstitial fluid (perhaps induced by an enhanced lymphatic return in exercising birds, although the relative contributions of this and other possible factors, including alterations in Starling forces across capillaries, are unknown). Some contribution to a reduced hematocrit could also result from shrinkage of erythrocytes in response to the elevated plasma osmolality; however, the change in hematocrit was not correlated with the change in plasma osmolality (on average, hematocrit decreased to a greater extent in winter, even though plasma osmolality increased less, Fig. 1), and so it seems likely that plasma volume was indeed expanded during flight.

The changes in cardiovascular variables discussed above, i.e. expanded plasma volume and decreased hematocrit, may be part of a suite of cardiovascular changes which function to ensure adequate oxygen delivery during flight. Expanded plasma volume may contribute to the maintenance of blood

pressure, while decreased hematocrit may reduce viscosity and thereby enhance blood flow to metabolically active tissues during exercise. Similarly, the lack of increase in GFR during flight, despite an increase in cardiac output of up to fivefold (Butler *et al.* 1977), suggests that renal blood flow is regulated during exercise in birds, as it is in mammals.

Interestingly, we found that the relative change in plasma volume (as indicated by changes in hematocrit) was correlated with the change in body water (assumed to equal the change in body mass) (Fig. 2). Moreover, only at high levels of water loss, i.e. in excess of 12% of body mass, was the expansion of plasma volume compromised. Expansion of plasma volume may be critical for birds to continue normal cardiovascular function (e.g. respiratory gas exchange and heat transfer) during flight. Thus, it is tempting to speculate that a limitation on the ability to maintain plasma volume in the face of increasing loss of body water could place a limit on the duration of bird flight, particularly in warm weather.

Plasma AVT concentration

Post-flight plasma AVT concentrations were 3–8 times the pre-flight levels and were in the range 70–100 pg ml⁻¹ (Table 1). Such high levels have been reported in some other species of birds only after severe dehydration (e.g. Goldstein and Braun, 1988; Stallone and Braun, 1986*a*; Nouwen *et al.* 1984). George *et al.* (1992) also found significantly elevated

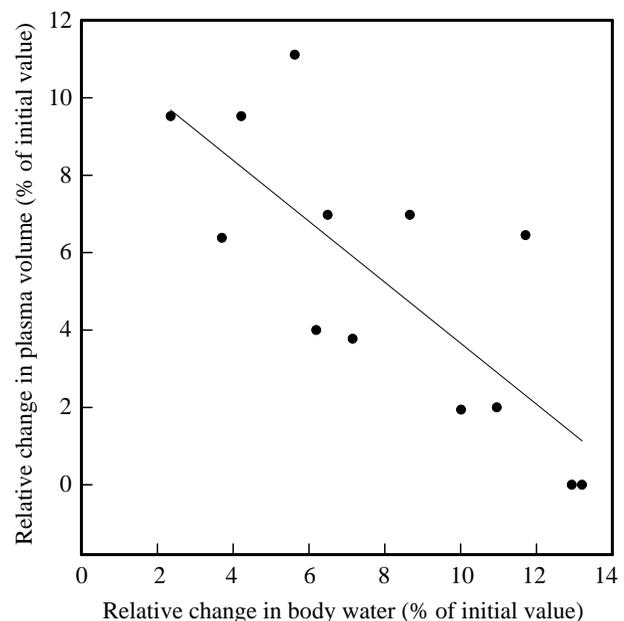


Fig. 2. Relationship between the estimated relative change in plasma volume (RPV) and relative change in body water (RBW) in free-flying tippler pigeons. RPV is given relative to an initial value of 100 and is estimated from the changes in hematocrit, i.e. 100 minus hematocrit (in %), assuming that changes in hematocrit are directly related to changes in plasma volume. RBW is calculated as the change in body mass relative to its initial value and assuming that body mass loss equals water loss during flight. $RPV = 12 - 0.76RBW$ ($N = 13$, $r^2 = 0.6$, $P = 0.002$).

plasma AVT levels in homing pigeons after flight ($18.5 \pm 0.4 \text{ pg ml}^{-1}$), although these were much lower than the post-flight AVT concentrations that we measured. The reasons for this difference might include the shorter flight duration of the homing pigeons in that study (1–1.5 h compared with more than 3 h in the present study), the lower ambient temperature (10°C compared with $23\text{--}25^\circ\text{C}$ in the present study) or differences in AVT regulation between different pigeon strains.

Apparently, flying pigeons experience rises in both plasma osmolality and in plasma volume. These signals would be expected to have opposite effects on the release of AVT (stimulation by increased osmolality, inhibition by increased plasma volume). However, typically, the release of AVT from the neurohypophysis is more sensitive to the former signal than to the latter (e.g. Stallone and Braun, 1986b), and the changes we measured during flight and at rest are consistent with this. The significant correlation between body mass loss (which in flying birds represents mainly water loss; Giladi, 1997) and post-flight AVT levels suggests that AVT concentration (along with plasma osmolality) could be a good indicator of the dehydration state of a flying bird.

In all flights, plasma osmolality and AVT levels increased, while hematocrit decreased, compared with pre-flight values. In examining the relationships between post-flight AVT concentration and plasma osmolality, hematocrit and the body mass loss that took place during flight, we found that only body mass loss was significantly correlated with AVT concentration. This inconsistency with the fact that AVT concentration is generally positively correlated with plasma osmolality (Stallone and Brown, 1986b) probably stems from the fact that we measured a very narrow range of plasma osmolality in post-flight birds. This made it difficult to discern any significant correlation between plasma osmolality and any other variable, including AVT concentration. Alternatively, it might reflect the action of different cues for the release of AVT during flight compared with rest (AVT release during flight might be more sensitive to water loss *per se* than at rest). For instance, in domestic fowl *Gallus domesticus*, release of AVT was triggered by heat stress without any change in plasma osmolality (Wang *et al.* 1989); the internal heat load incurred by a flying bird could have a similar effect.

In addition to its antidiuretic effect, AVT has other functions which are conceivably important for flying birds. These include promotion of lipid catabolism (John and George, 1986), increasing heart rate and respiratory frequency, and even induction of a hypothermic response (John and George, 1992); therefore, AVT release during flight may be advantageous for reasons other than osmoregulation.

Kidney function

Consistent with the reduction in body mass, the increase in plasma osmolality and the elevation of plasma AVT level, fractional water reabsorption showed a tendency to be higher in flying birds in both winter and summer; urine flow rate showed the opposite tendency. Since pre-flight variability in

both urine flow rate and fractional water reabsorption in both seasons was very high, the likelihood of establishing statistically significant differences between pre- and post-flight values was lower. However, because the changes in these variables were of the same order of magnitude in both seasons, we presume that the clear trends we see in the data are real, despite the lack of statistical significance. The summer reduction in GFR may reflect the more severe water loss and the greater rise in plasma osmolality that occurred during summer flight (Fig. 1). It could also, in part, reflect the initial (pre-flight) physiological condition of the birds in the two seasons (different GFR and urine osmolality, as discussed above).

We note also that factors other than dehydration-induced release of AVT could influence renal function during exercise. In mammals, for example, the activity of the adrenergic sympathetic renal nerves increases during exercise (O'Hagan *et al.* 1993) and may contribute to the regulation of renal hemodynamics and filtration. Likewise, norepinephrine contributes to renal osmoregulation in birds (Jungbluth *et al.* 1994), although its role in avian exercise has not been examined.

Urinary water loss relative to water loss by other avenues

The urine flow rates that we measured can be placed into the context of overall patterns of water loss. Water efflux rates in resting pigeons from the same stock as those used in the present study were approximately 1.5 ml h^{-1} at 14°C and 2.2 ml h^{-1} at 19°C (Giladi, 1997). Of this, approximately 10–20% (0.3 ml h^{-1}) was excretory (Giladi, 1997). In comparison, the urine flow rates that we measured in resting birds ($>1.5 \text{ ml h}^{-1}$) exceeded the excretory water losses measured by Giladi (1997) by several-fold. This comparison suggests that, as in a number of other species (e.g. Goldstein *et al.* 1986; Goldstein and Braun, 1986), a significant proportion of the urine is reabsorbed in the cloaca of the pigeon at rest.

Mean excretory water loss in flying pigeons was approximately 0.53 ml h^{-1} at 14°C and 0.49 ml h^{-1} at 19°C (Giladi, 1997). The urine flow rates that we measured in flying birds (approximately 0.7 ml h^{-1}) exceed the excretory losses measured by Giladi (1997) by 30–40%, suggesting a lesser, but still significant, role for post-renal reabsorption of urinary water during flight. The tendency towards lower rates of post-renal reabsorption during flight could be a consequence both of reduced urine flow rate (which may limit the reflux of urine into the absorptive segments of the lower intestine; Brummermann and Braun, 1995) and of the higher urine osmolality (which imposes an osmotic gradient opposing reabsorption; Goldstein *et al.* 1986). It is also worth noting that, during both rest and flight in pigeons, approximately 90% of the total water loss is evaporative (Giladi and Pinshow, 1996).

We conclude that the mechanisms of water conservation in flying birds are qualitatively very similar to those that function during dehydration and that conservation of excretory water may be important in maintaining fluid balance in flying pigeons.

We especially thank Nigel Adams, Gilead Michaeli and Leonard Gannes for their assistance during experiments. Eyal Shani provided invaluable help maintaining and training birds. We thank Dr William H. Karasov for constructive criticism. Support for this study was provided by United States–Israel Binational Science Foundation grant 93-00232. This is paper number 238 of the Mitrani Center for Desert Ecology.

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