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Gerson AR, Guglielmo CG. House sparrows (*Passer domesticus*) increase protein catabolism in response to water restriction. *Am J Physiol Regul Integr Comp Physiol* 300: R925–R930, 2011. First published January 19, 2011; doi:10.1152/ajpregu.00701.2010.—Birds primarily rely on fat for energy during fasting and to fuel energetically demanding activities. Proteins are catabolized supplemental to fat, the function of which in birds remains poorly understood. It has been proposed that birds may increase the catabolism of body protein under dehydrating conditions as a means to maintain water balance, because catabolism of wet protein yields more total metabolic and bound water ($0.155 \cdot H_2O^{-1} \cdot kJ^{-1}$) than wet lipids ($0.029\ g \cdot H_2O^{-1} \cdot kJ^{-1}$). On the other hand, protein sparing should be important to maintain function of muscles and organs. We used quantitative magnetic resonance body composition analysis and hygrometry to investigate the effect of water restriction on fat and lean mass catabolism during short-term fasting at rest and in response to a metabolic challenge (4-h shivering) in house sparrows (*Passer domesticus*). Water loss at rest and during shivering was compared with water gains from the catabolism of tissue. At rest, water-restricted birds had significantly greater lean mass loss, higher plasma uric acid concentration, and plasma osmolality than control birds. Endogenous water gains from lean mass catabolism offset losses over the resting period. Water restriction had no effect on lean mass catabolism during shivering, as water gains from fat oxidation appeared sufficient to maintain water balance. These data provide direct evidence supporting the hypothesis that water stress can increase protein catabolism at rest, possibly as a metabolic strategy to offset high rates of evaporative water loss.

metabolic water; total evaporative water loss; magnetic resonance body composition analysis

BIRDS HAVE AN EXCEPTIONAL ability to rapidly mobilize and catabolize fat to fuel metabolically demanding activities such as flight or thermogenesis (21, 25, 36, 46). Supplemental to fat catabolism, it has become apparent that protein in lean tissue is also catabolized during flight, thermogenesis, and at rest (3, 7, 25, 27, 28, 31, 42, 45). Protein is primarily catabolized for energy during phase III of fasting when fat and glycogen stores have been depleted (13), but protein catabolism during phase I of fasting, while an animal still has sufficient energy stores remaining, may be in response to other physiological factors.

Catabolism of protein during flight in birds has been documented through gravimetric changes in muscles and organs (3, 5, 32, 42) and through changes in plasma metabolites such as uric acid (16, 22, 26, 27, 43, 49). Since there is no storage tissue for protein as there is for fat (adipocytes) or carbohydrates (liver and muscle glycogen), protein is used directly from muscles and organs with possible negative consequences to flight performance in the case of muscle catabolism or nutrient absorption and processing in the case of organ catabolism.

There has been much discussion about the possible role for this seemingly maladaptive phenomenon (3, 7, 11, 12, 14, 25, 29, 31–33, 37, 42). Protein catabolism may be necessary for gluconeogenesis or for the anaplerosis of tricarboxylic acid (TCA) cycle intermediates, both of which may be necessary during sustained fat catabolism (14, 25). The breakdown of protein could also aid in the maintenance of water balance under dehydrating conditions especially in uricotelic animals, where the excretion of nitrogenous wastes requires less water than in ureotelic animals (25, 29, 50). For the same amount of energy released, catabolism of wet protein results in the release and production of $0.155\ g \cdot H_2O^{-1} \cdot kJ^{-1}$, approximately five-times more bound and metabolic water than the catabolism of fat ($0.029\ g \cdot H_2O^{-1} \cdot kJ^{-1}$) (25). Thus, protein may serve as a source of endogenous water to offset water losses, while additionally providing the metabolites necessary for gluconeogenesis and anapleurosis of TCA cycle intermediates (29).

Many of the studies documenting substantial lean mass losses of birds were performed on trans-Saharan migrants or after multiday nonstop flights in shorebirds, where water stress is possible (3, 4, 7, 12, 28, 29, 33). Although there is evidence that long-term dehydration can increase protein catabolism in humans and Richardson's ground squirrels (*Spermophilus richardsonii*) (6, 8, 23), to date there are no studies showing a direct reduction in the total lean mass of an animal due to acute dehydration. Thus, we postulate that the amount of lean mass catabolized may not necessarily depend on energetic demands and may instead be a response to water deficit or other stressors. If this is the case, it is expected that water-restriction will increase lean mass catabolism, ultimately resulting in maintenance of water balance due to endogenous water gains.

Until recently, it has proven difficult to accurately measure the effects of water restriction on body composition over time within an individual (35, 38). Techniques such as heavy water dilution for estimating lean mass rely on total body water and can be confounded by manipulation of water balance (44). Catabolism of protein in a uricotelic animal results in respiratory quotients (CO_2 produced ÷ O_2 consumed) very similar to those of fat catabolism (31), precluding the use of respirometry for the determination of fuel mixture including a protein component. Destructive body composition analysis requires a large number of individuals, and would lack the power of a repeated-measures design. Therefore, in this study we used a quantitative magnetic resonance (QMR) body composition analyzer, which accurately and noninvasively measures lean mass, fat mass, and total body water in unanaesthetized animals (47, 48). Changes in body composition were monitored longitudinally in individual birds throughout the course of an

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18-h water restriction at rest, followed by a 4-h period of elevated metabolic rate (shivering). Our goal was to investigate whether water balance status affects the rate of lean mass catabolism during rest and simulated endurance exercise in house sparrows (*Passer domesticus*). For birds to maintain water balance during the resting phase of this experiment, additional endogenous water production should be necessary from lean mass catabolism. However, during the shivering phase of the experiment high metabolic rates will result in elevated metabolic water production primarily from fat. Depending on the rate of ventilatory water loss during shivering, net metabolic water gains could reduce or preclude water required from lean mass catabolism.

MATERIALS AND METHODS

Animal care. Male house sparrows (*P. domesticus*) were caught by using mist nets during March of 2008 near the University of Western Ontario campus (London, ON, Canada). Birds were moved to the animal care facility within 1 h of capture where they were weighed, individually color banded, and placed individually in 40 cm × 45 cm × 45 cm cages with water and a diet consisting of a mixture of millet seed and Mazuri Small Bird diet. Birds were maintained on a 12:12-h light-dark (lights on at 06:00) cycle at 24°C for the duration of the experiment. Birds were kept for a minimum of 2 wk before the experiment began. All animal care protocols followed the Canadian Council on Animal Care guidelines and were approved by the University of Western Ontario Council on Animal Care and the Animal Use Subcommittee (protocol no. 2006-011-04).

Experimental protocol. Sparrows were randomly assigned to either a water-restricted (WR; $n = 8$) or control (CT; $n = 7$) group, which were housed individually in adjacent cages, thus controlling for minor fluctuations in light and disturbance in the housing facility. These pairs of birds followed identical routines throughout the experimental period. Each experimental day between 15:30 and 17:30, one CT bird and one WR bird were weighed, scanned in duplicate, using QMR (initial scan), and returned to their cages. Each replicate QMR scan lasted ~90 s. After the initial scan, water was removed from the cage of the WR bird. At 07:00 the following morning, food was removed from the cages of the WR and CT birds. At 10:00 birds were assumed to be postabsorptive, and were placed in identical adjacent 1-liter respirometry chambers maintained at 24°C for a period of 2 h to determine resting metabolic rate (RMR) and resting rates of total evaporative water loss (see below), at this time the WR birds had experienced 16–18 h without access to free water. After 2 h at 24°C, the birds were scanned in duplicate using QMR (before shivering), blood was sampled from the right brachial vein (rest), and the birds were then placed in identical individual 1-liter respirometry chambers that had been precooled to initiate a 4-h shivering trial at 5°C. Shivering metabolic rate (SHMR) and rates of total evaporative water loss were measured (see below). At the conclusion of the shivering trial, birds were scanned in duplicate a third time using QMR (postshivering), and a final blood sample was taken from the left brachial vein (shivering). The bottom of each metabolic chamber was lined with aluminum foil and all droppings were collected and immediately frozen at −30°C after the resting period and the shivering trial. Each individual bird was used only once during the course of the experiment.

QMR. QMR body composition analysis has been shown to be extremely accurate and precise, and the principle of the methods has been described elsewhere (36, 45, 49, 50). The instrument we used was specifically designed for use with small birds (model MRI-B; Echo Medical Systems, Houston, TX). Our validation studies with house sparrows indicate that fat, wet lean, and total body water are measured with precisions (coefficient of variation) of 3%, 0.5%, and 3%, respectively, and relative accuracies of ±11%, ±1%, and ±2%, respectively (Guglielmo CG, Gerson AR, McGuire LP, and Seewagen CL, unpublished data). Overall changes in body mass, lean mass, and fat mass were compared between the WR and CT groups by using a repeated-measures general linear model (GLM) with initial mass as a covariate. Between treatment effects for each experimental interval (initial-to-before shivering and before shivering-to-postshivering) were assessed by comparing the differential in body mass, lean mass, and fat mass for each interval using GLM (SPSS version 17.0).

Respirometry, hygrometry, and estimated water budgets. Flow through respirometry was used to measure RMR and SHMR simultaneously with rates of evaporative water loss. Incurrent air was scrubbed of CO_2 and water vapor using soda lime and Drierite, respectively. All four respirometry chambers were well sealed and received constant flow of ~600 ml/min (measured after the chambers with a 840-liter mass flow meter; Sierra Instruments, Monterey, CA). Excurrent air was subsampled at a rate of 150 ml/min through a H_2O analyzer (Licor LI-7000) after which air passed through a Drierite column to the CO_2 (cat. no. CA-2A; Sable Systems Las Vegas, NV) and oxygen gas analyzers (Sable Systems FC-1B) with CO_2 and H_2O scrubbing between the two gas analyzers. Gas analyzers were calibrated with a certified standard (20.9% O_2-2.0% CO_2 balanced with N_2; Praxair, London, ON, Canada). Multiplexing allowed measurement of each chamber in 30-min intervals, with a 10-min baseline measurement every hour. Two respirometry chambers were placed in a temperature-controlled cabinet maintained at 24°C (model PTC-1; Sable Systems), while another two chambers were maintained at 2–5°C in a Styrofoam cooler lined with copper tubing that was connected to a water bath (Lauda E100) circulating −8.0°C propylene glycol. This circulating temperature was most effective at maintaining chamber temperature between 2°C and 5°C during the shivering trial. All instruments were connected to an analog-to-digital converter (model UI-2; Sable Systems), which was connected to a laptop computer. Data collection and analysis were done using Expedata software (Sable Systems). Fractional concentrations of O_2 and CO_2 were lag corrected and $\dot{V}O_2$ (ml/min), $\dot{V}CO_2$ (ml/min), and $\dot{V}H_2O$ (mg H_2O/h) were calculated from the mean for the final 20 min of each 30-min sampling period for each channel using equations 11.1, 11.6, and 11.9, respectively, from (34), assuming that 1 ml of water vapor is equivalent to 0.803 mg H_2O (34). $\dot{V}H_2O$ at rest and during shivering was extrapolated over time to estimate total water lost during those periods. Endogenous water production (sum of metabolic water and water liberated from catabolism of lean mass) was calculated from the values in Jenni and Jenni-Eiermann (25), and the change in lean and fat mass measured by QMR. Estimated total evaporative water loss and endogenous water production were compared between treatments using Student's *t*-test. Upon analysis of the respirometry data, the RQ values were unrealistically low, and it was determined that the fuel cell in the oxygen analyzer had expired; thus $\dot{V}O_2$ data was discarded. $\dot{V}CO_2$ and $\dot{V}H_2O$ during resting and shivering trials was compared between treatment groups using Student's *t*-test.

Uric acid and osmolality determination. Uric acid was determined by end point assay (uric acid 20R/30R kit, Wako) as in Tsahar, et al. (49) for both plasma and droppings. Droppings were weighed and dried to a constant mass at 45°C. Dried excreta was then ground using a small glass mortar and Teflon pestle and dissolved 120-fold (wt/vol) in 0.1 M glycine buffer, pH 9.3 for analysis; plasma was analyzed undiluted. Excreted uric acid was only compared between treatments during the resting period due to a low number of dropping samples during the shivering trial. Plasma osmolality was measured in 10 μl of plasma using a Wescor Vapro 5520 vapor pressure osmometer calibrated as per the manufacturers instructions. Plasma uric acid and osmolality from rest and shivering blood samples were compared between WR and CT birds using repeated-measures GLM. Excreted uric acid was compared between treatments using *t*-test (SPSS version 17).

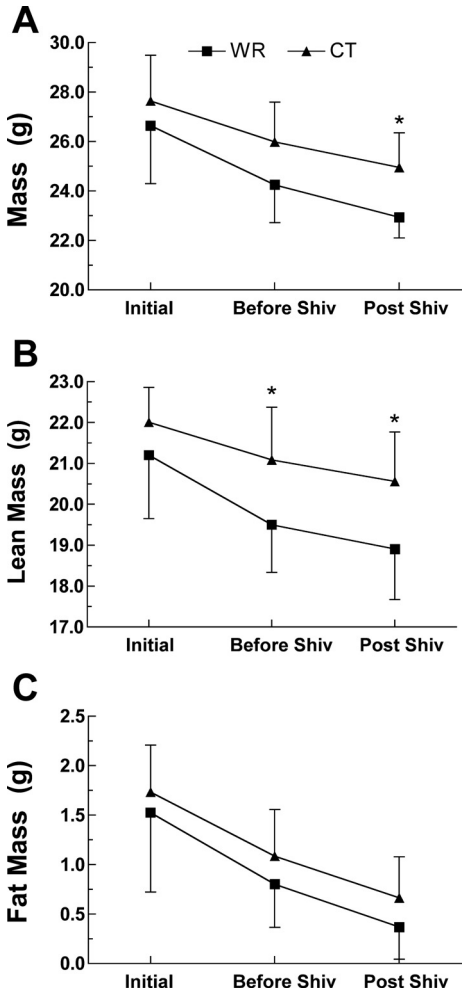

Fig. 1. *A*: water restriction (WR) resulted in greater mass loss overall. *B*: lean mass loss in WR birds was greater during the resting period and overall. *C*: no significant differences in fat mass losses were evident between WR and control (CT) birds. Shiv, shivering. WR: $n = 8$, CT: $n = 7$. Values are means ± SD. *Significant difference between WR and CT at $P < 0.05$.

RESULTS

Body composition. All birds lost mass throughout the experiment (Fig. 1*A*, $F_{2,20} = 124.769$, $P = 0.001$), but WR birds lost on average 1.01 g (4.3%) more than CT birds (Fig. 1*A*, $F_{1,10} = 6.785$, $P = 0.026$). There was no significant difference in initial mass between treatments (*t*-test: $t = 1.150$, DF = 13, $P = 0.271$), and most mass loss occurred during the resting period for both WR and CT birds. Although mass loss during this time was 0.73 g (3.0%) greater in WR animals, mass loss at rest was not significantly different between treatments ($F_{1,13} = 2.811$, $P = 0.117$). The greater mass loss in WR animals was a result of an additional 0.85 g (4.3%) of lean mass loss overall ($F_{1,12} = 21.372$, $P = 0.001$). The majority (74.02%) of the lean mass loss occurred between the initial and before shivering time points, and during this time WR birds lost significantly more lean mass than CT birds (Fig. 1*B*, $F_{1,13} = 5.435$, $P = 0.036$); there was no significant difference between treatments in lean mass loss during shivering ($F_{1,13} = 1.410$, $P = 0.256$). No significant differences in fat mass losses were evident between WR and CT birds for either the overnight interval ($F_{1,13} = 0.230$, $P = 0.639$) or during the shivering trial ($F_{1,12} = 2.98$, $P = 0.108$) (Fig. 1*C*).

Table 1. *$\dot{V}CO_2$ and $\dot{V}H_2O$ at rest and during shivering in water restricted (WR) and control (CT) birds*

	WR		CT	
	Rest	Shivering	Rest	Shivering
$\dot{V}CO_2$, ml $CO_2 \cdot min^{-1} \cdot g^{-1}$	2.00 (0.28)	5.02 (1.68)*	1.88 (1.35)	4.54 (2.21)*
$\dot{V}H_2O$, ml $H_2O \cdot min^{-1} \cdot g^{-1}$	3.89 (0.77)	5.40 (1.22)	4.33 (1.64)	4.85 (1.01)

Values are means (SD). No significant differences were found between WR and CT. Shivering resulted in significantly elevated $\dot{V}CO_2$ over resting values within each treatment group. *$P < 0.05$.

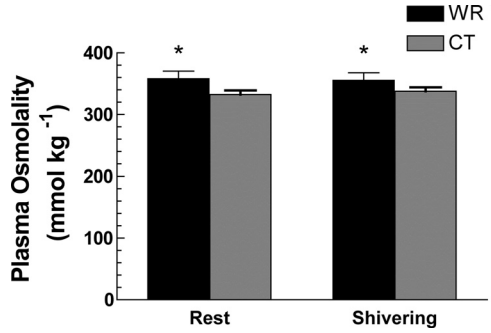

Fig. 2. Plasma osmolality was significantly elevated by WR both at rest, and postshivering (WR: $n = 8$, CT: $n = 7$). Values are means ± SD. *Significant differences between treatments at $P < 0.05$.

Respirometry. No significant differences were found between WR and CT groups in $\dot{V}CO_2$ ($t = 0.320$, DF = 13, $P = 0.754$) or $\dot{V}H_2O$ ($t = 0.675$, DF = 13, $P = 0.512$) at rest or during shivering $\dot{V}CO_2$ ($t = -0.495$, DF = 14, $P = 0.628$); $\dot{V}H_2O$ ($t = -1.421$, DF = 14, $P = 0.177$). Shivering resulted in a significant increase in $\dot{V}CO_2$ over resting in both treatments (WR: $t = -7.169$, DF = 14, $P < 0.001$; CT: $t = -2.259$, DF = 14, $P < 0.001$; Table 1).

Plasma osmolality and uric acid. Plasma osmolality at rest was 7.3% higher in WR birds (Fig. 2: $F_{1,11} = 20.080$, $P = 0.001$), and was 4.97% higher in WR birds after the shivering trial (Fig. 2: $F_{1,11} = 9.639$, $P = 0.010$). Plasma concentrations of uric acid were also elevated in the WR group at rest (Fig. 3: $F_{1,11} = 15.384$, $P = 0.002$). However, postshivering, there was no significant difference in plasma uric acid between the WR and CT samples (Fig. 3: $F_{1,11} = 1.963$, $P = 0.192$). Both WR

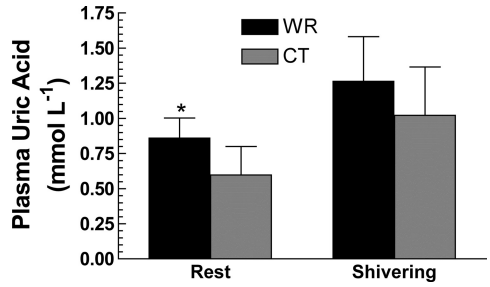

Fig. 3. Plasma concentrations of uric acid were elevated in the WR group at rest, but not after shivering. Both treatments had increased plasma uric acid postshivering (WR: $P = 0.005$, CT: $P = 0.013$). Values are means ± SD. *Significant differences between treatments at $P < 0.05$.

Table 2. *Uric acid concentration, moisture, and total uric acid lost in excreta at rest during respirometry from WR and CT birds*

Rest	Uric Acid, mg/g	% Moisture	Uric Acid Lost, mg
WR	418.01 (66.61)	5.97 (6.09)	344.01 (65.78)
CT	309.91 (135.20)	9.74 (8.06)	261.62 (116.28)
P value	0.080	0.147	0.108

Values are means (SD). No significant differences were evident between treatments.

and CT animals experienced an increase in plasma uric acid with shivering (Fig. 3: WR: $t = -3.984$, DF = 7, $P = 0.005$; CT: $t = -3.736$, DF = 5, $P = 0.013$). Treatment did not affect excreta uric acid concentration ($t = -1.60$, DF = 6, $P = 0.080$), water content ($t = 0.832$, DF = 7, $P = 0.432$), nor the total amount of uric acid lost in excreta during the resting period ($t = -1.379$, DF = 6, $P = 0.108$), although the uric acid concentration of the excreta, as well as the total amount of uric acid lost, tended to be higher in WR birds (Table 2).

Water budgets. WR sparrows produced significantly more endogenous water from the catabolism of lean mass at rest ($t = -2.489$, DF = 14, $P = 0.026$), but not during the shivering trial ($t = 0.93$, DF = 14, $P = 0.927$), and they tended to produce more water during the entire experiment from lean mass catabolism ($t = -2.089$, DF = 13, $P = 0.057$). As a result, WR birds produced more total endogenous water (water from lean and fat) at rest ($t = -1.94$, DF = 14, $P = 0.036$) than CT birds. During shivering, CT birds had greater total endogenous water production ($t = 2.27$, DF = 12, $P = 0.020$). There were no significant differences in estimated total evaporative water loss between treatments for either the resting ($t = 1.033$, DF = 13, $P = 0.320$) or shivering ($t = -0.69$, DF = 13, $P = 0.946$) periods. At rest, WR birds maintained positive net water balance, which was significantly higher than CT birds, where net water balance was negative ($t = -2.129$, DF = 9, $P = 0.033$). During shivering, metabolic water production exceeded water losses regardless of treatment, but CT birds had greater gains than WR birds ($t = 2.20$, DF = 13, $P = 0.023$). Total body water as a percent of body mass did not change significantly between resting and shivering periods (rest: $t = -0.045$, DF = 11, $P = 0.482$; shivering: $t = -0.876$, DF = 7, $P = 0.204$) (Table 3).

DISCUSSION

This is the first study, to our knowledge, to directly test the hypothesis that birds preferentially catabolize protein as a means to liberate endogenous water under conditions of water stress. The elevated lean mass loss as a result of acute water restriction as shown by the direct measurement of body composition over time within individuals represents clear support for this hypothesis. Although this hypothesis has been proposed mainly as a strategy for long distance migratory flight (25, 29), this experiment serves as a proof of concept that a physiological mechanism exists whereby water balance status can influence fuel mixture. Whereas a greater reduction in lean mass occurred only at rest in the WR group, it is important to note that during shivering, metabolic water production far exceeded water losses; consequently, additional water production from lean mass was unnecessary.

The strongest evidence for accelerated lean mass catabolism as a result of water restriction comes from the QMR data, which was corroborated by the elevated plasma uric acid levels. Changes in body composition coupled with $\dot{V}H_2O$ allowed the estimation of water budgets for each phase of this experiment, which indicate that higher rates of lean mass catabolism in the WR birds resulted in endogenous free water gains sufficient enough to offset evaporative losses at rest. During shivering, metabolic rate was elevated ~2.5-fold regardless of treatment. This elevation in metabolic rate was primarily fueled by fat catabolism, and the relative contribution from lean was similar for each treatment. Since water balance was maintained during the resting period, at the expense of lean mass in the WR birds, elevated lean mass catabolism during shivering was unnecessary for the WR birds.

Birds typically maintain plasma volume during water restriction (10), discounting the possibility that elevated osmolality and uric acid were simply a product of reduced plasma volume, rather than an increase in the actual metabolites responsible. In fact, elevated plasma osmolality may be a response to dehydration that facilitates the maintenance of plasma volume by favoring the movement of intracellular water to blood vessels down the osmotic gradient, thus resulting in cellular dehydration (1). There is evidence indicating hyperosmolality alone may influence cellular metabolism in mammals, ultimately resulting in elevated protein catabolism (6, 23). Whether this mechanism exists in birds has yet to be explored. The resulting

Table 3. *Estimated water budgets for WR and CT treatment groups throughout an 18- to 20-h dehydration period followed by a 4-h shivering trial*

	Rest		Shivering	
	WR	CT	WR	CT
Total evaporative water loss, ml	1.85 (0.42)	2.20 (0.85)	0.554 (0.11)	0.550 (0.11)
Endogenous water production, lean, g	1.40 (0.41)*	0.769 (0.59)	0.487 (0.11)	0.567 (0.15)
Total endogenous water production, g	2.19 (0.79)*	1.48 (0.68)	0.964 (0.14)*	1.14 (0.17)
Net water balance, g	0.348 (0.54)*	−0.443 (1.43)	0.409 (0.18)	0.598 (0.15)
Body water, % of M_b	63.07 (2.32)	63.00 (3.91)	64.41 (3.12)	62.93 (2.52)

Values are means (SD). Total endogenous water production was calculated from changes in body composition, assuming 0.155 $g \cdot H_2O^{-1} \cdot kJ^{-1}$ for lean mass and 0.029 $g \cdot H_2O^{-1} \cdot kJ^{-1}$ for fat as in Ref. 25. Net water balance is the difference between total evaporative water loss and total endogenous water production; CT birds had access to water during the resting period. Body water, % of M_b (metabolic states) is the quantitative magnetic resonance value for total body water divided by total body mass for the before and after shivering time points. *Significant differences ($P < 0.05$) between treatment groups but within metabolic states.

endogenous water production may alleviate cellular dehydration, while amino acids and peptides may bring intracellular osmolality toward equilibrium with the interstitial fluid and plasma. This mechanism would not only liberate water, but would reduce the osmotic gradient between the intra- and intercellular compartments. Taking this into account, the decrease in plasma osmolality during the shivering trial in the WR birds could be a product of water gains due to higher metabolism overall and thus greater metabolic water production, resulting in the expansion of plasma volume during the shivering period.

Responses of birds to extended dehydration typically include reduced glomerular filtration rate and greater tubular water reabsorption, resulting in production of more concentrated urine (19, 20). Although no significant differences were found between treatments, the trends in uric acid excretion are consistent with this response. However, the degree of the response is subtle, perhaps because the period of water restriction in the current experiment was relatively short. Birds are uricotelic and utilize extrarenal water reabsorption in the colon to minimize excretory water losses (20). For this reason, birds may be distinct from mammals in their ability to benefit from a protein-for-water strategy so long as urine does not reach a concentration where extrarenal absorption is repressed (19). However, highly concentrated urine is unlikely in the present study due to the relatively short water restriction and the maintenance of water balance from elevated protein catabolism. Body water as a percent of body mass did not change throughout the experiment, but it has been noted that the amount of water relative to body mass is not a good indicator of water stress or dehydration (29).

Due to the limited amount of plasma available, other analyses were not possible in this study. The response of arginine vasotocin, prolactin, aldosterone, or corticosterone to water restriction could have provided insight into possible control of fuel selection during water restriction. However, this study did not differ substantially in terms of magnitude or duration of water restriction from many other studies that have thoroughly investigated the hormonal response to acute water restriction in birds at rest and during exercise (2, 17, 19, 39). Corticosterone does affect fuel use in birds, and would be the likely hormone responsible for elevated lean mass catabolism. However, experimental design must incorporate careful control of handling and other stressors during the experiment for measurements of corticosterone to be meaningful.

The protein-for-water phenomenon could have broad implications during many life history stages of birds, including breeding and migration, especially in light of projected changes in climate (24). The use of protein for water may help explain recent evidence that birds actually fly under conditions unfavorable to water balance (41), taking advantage of favorable winds, and possibly maintaining water balance at the expense of protein. If water stress during migratory flight or stopover refueling results in additional lean mass catabolism, refueling rates may be reduced (40), leading to increased stopover duration and delaying arrival on the breeding grounds (9).

Perspectives and Significance

Since much of the discussion surrounding the hypothesis that protein can be stored and used for water production has focused on migratory flight, it would seem logical to design experiments to test the possibility that water balance may influence the proportions of fat and lean mass utilized during flight. To this end, we feel that the present study utilizes a simple yet informative suite of minimally invasive techniques that could be implemented to study the possible effects of environmental conditions on fuel mixture utilization in avian flight. It would then be interesting to modify existing fuel use models for bird flight to examine the possible consequences of dehydration to flight range in terms of changing fuel mixture (11, 12, 29, 30). It should be noted that due to the very high metabolic rates experienced during flight, a vast amount of metabolic water is produced from the catabolism of fat alone. Whether this water production is balanced by water loss depends entirely on the ambient conditions experienced during flight. Rates of water loss are constant across temperatures during flight, until a threshold temperature is reached above which evaporative water loss increases with temperature (15, 18). This threshold temperature has been identified to be ~20°C for both rose-colored starlings (*Sturnus roseus*) and pigeons (*Columbia livia*) (15, 18). Flying at temperatures above this threshold may result in greater lean mass utilization for water in flight. Future studies should investigate this phenomenon in resting and exercising animals to more fully understand the mechanisms involved in the control of lean mass catabolism as well as the osmoregulatory consequences of lean mass catabolism during water restriction at both the cellular and whole animal levels.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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