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RESEARCH ARTICLE

Increased fat catabolism sustains water balance during fasting in zebra finches

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ABSTRACT

Patterns of physiological flexibility in response to fasting are well established, but much less is known about the contribution of water deprivation to the observed effects. We investigated body composition and energy and water budget in three groups of zebra finches: birds with access to food and water, food-deprived birds having access to drinking water and food-and-water-deprived birds. Animals were not stimulated by elevated energy expenditure and they were in thermoneutral conditions; thus, based on previous studies, water balance of fasting birds was expected to be maintained by increased catabolism of proteins. In contrast to this expectation, we found that access to water did not prevent reduction of proteinaceous tissue, but it saved fat reserves of the fasting birds. Thus, water balance of birds fasting without access to water seemed to be maintained by elevated fat catabolism, which generated 6 times more metabolic water compared with that in birds that had access to water. Therefore, we revise currently established views and propose fat to serve as the primary source for metabolic water production. Previously assumed increased protein breakdown for maintenance of water budget would occur if fat stores were depleted or if fat catabolism reached its upper limits due to high energy demands.

KEY WORDS: Protein sparing, Metabolic water, Body composition, Energy budget, *Taeniopygia guttata*

INTRODUCTION

Water plays a vital role in all body processes, from biochemical reactions, to regulation of osmolality and thermoregulation. Thus, availability of water restricts the physiological functions and habitat use of an organism. For instance, in birds, shortage of water impairs reproductive investment (e.g. Giuliano et al., 1998; Prior et al., 2013) and may set limits to potential habitat, flight range or flight altitude during migration (e.g. Hohtola et al., 1994; Klaassen, 1996, 2004; McKechnie and Wolf, 2010; McNabb, 1969). However, shortage of water may to some extent be overcome by metabolic water production.

There are two main sources of metabolic water: catabolism of fat and catabolism of protein, which differ in energy content and amount of water that can be liberated. Burning lipids yields only 0.029 g water kJ^{-1} expended from wet mass (Jenni-Eiermann and Jenni, 2012). Yet, catabolism of fat stored in adipose tissue contributes to maintain water balance under conditions of elevated metabolic rate, such as in the course of flight (Gerson and Guglielmo, 2011a) or shivering (Gerson and Guglielmo, 2011b), during which fat is the main source of energy. Burning protein yields 0.155 g water kJ^{-1} expended from wet mass (Jenni-Eiermann and Jenni, 2012). A certain level of protein catabolism takes place all the time, as part of physiological protein turnover (Bauchinger and McWilliams, 2010), and it is also required for the process of fat breakdown (Jenni-Eiermann and Jenni, 1991). In animals experiencing high energy expenditure, even a small amount of additionally catabolised proteinaceous tissue can generate water required to maintain the water balance (Gerson and Guglielmo, 2011a,b).

Less is known about endogenous water production by animals under routine maintenance conditions. Two previous studies suggested that fasting animals that are not challenged by physical activity sustain water balance by preferentially catabolising proteins over fat (Bintz and Strand, 1983; Gerson and Guglielmo, 2011b). However, in both studies, the manipulation of water availability was confounded with access to food. In the study on Richardson ground squirrels *Spermophilus richardsonii*, water availability was achieved by feeding the animals with celery, which is highly hydrated but contains a low amount of digestible matter (Bintz and Strand, 1983). Thus, the study compared anabolic animals (no food, but water through celery) with catabolic animals (no food, no celery). In the study on the house sparrow *Passer domesticus*, experimental birds had access to food for 1 h in the morning before their body content was measured using quantitative magnetic resonance (Gerson and Guglielmo, 2011b). Thus, at least for some time during manipulation of water availability, the animals could have been in an anabolic state. Therefore, the main conclusion from those studies, namely that water restriction leads to increase protein catabolism, requires revisiting in individuals in a true catabolic state.

Our study aimed at investigating the effects of water availability on gross body composition and overall energy and water budget of birds. As a model species, we used zebra finches, *Taeniopygia guttata* Reichenbach 1862, which modulate physiology and behaviour in response to immediate environmental conditions, including food and water deprivation (e.g. Lynn et al., 2010; Rashotte et al., 2001). We challenged the animals by food deprivation, but did not induce any physiological conditions that would require elevated energy expenditure, and maintained them under thermoneutral conditions. We predicted that food-restricted animals with access to drinking water will differ in the use of endogenous resources compared with food-and-water-deprived animals. If protein catabolism indeed contributes to water management, than individuals with access to water should maintain more lean dry mass. Consequently, because the lean fraction of body mass contributes to the basal metabolic rate (BMR; Chappell et al., 1999), they should also have higher whole-animal and mass-specific BMR compared with birds without access to drinking water.

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Received 15 February 2016; Accepted 14 June 2016

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MATERIALS AND METHODS

Experimental setup

Experimental birds originated from the captive population maintained at the Institute of Environmental Sciences since 2000. The birds were 8–24 months old. For 2 months prior to the experiment, the birds were kept in single-sex groups of 9–11 birds in cages measuring 60×70 cm and 40 cm high. Throughout the study, the photoperiod was 12 h:12 h light:dark (lights on at 08:00 h) and the temperature was 30±1°C (thermoneutral conditions for zebra finches). They were fed with a standard mixture of several species of millet seeds (Megan, Poland); water, cuttlefish bone and grit were also available. During this period, all the birds were exposed to unpredictable food and water deprivation periods, two of which lasted from 08:00 h to 14:00 h and two from 08:00 h to 17:00 h. Altogether, the birds were captured 13–15 times and their body mass was measured. Our aim was to accustom individuals to being captured and to food deprivation. None of the birds died during training or during the experiment itself. Birds were recruited from this stock to the experimental groups over seven consecutive days (which were controlled for as a random effect).

The day before the experiment, each bird was captured from its cage and placed alone or with another bird from the same experimental group in a new cage in which a feeding regime was imposed at 19:40 h (lights off at 20:00 h). Their body mass (hereafter 'initial body mass') was measured to the nearest 0.01 g (Radwag PS3500/C/1). At this point, the birds were randomly assigned to three groups: (i) *ad libitum* access to food and water, 7 males and 5 females; (ii) *ad libitum* access to water but deprived of food for 24 h, 7 males and 5 females; (iii) deprived of both food and water for 24 h, 6 males and 6 females. Birds assigned to the experimental groups did not differ in mean age ($F_{2,33}$=1.54, P=0.23), tarsus length ($F_{2,33}$=2.14, P=0.13) and initial body mass ($F_{2,33}$=0.01, P=0.99).

The following day, the birds were weighed at 08:00 h and at 14:30 h, after which they were placed without water and food into respirometry chambers. Their metabolic rate was measured until 18:00 h (see below). Each bird was then weighed and blood samples were taken, before it was killed by decapitation between 18:05 h and 19:59 h (the exact time did not differ between groups). Within 10–12 min, the following organs and tissues were dissected: heart, brain, liver, kidneys, pancreas, small intestine, gizzard, left flight muscle and leg muscle; these were weighed immediately to the nearest 0.00001 g (Radwag XA110/2X).

The remaining carcass and organs were dried to constant mass in a freeze dryer (Christ Beta 1-8 LD plus Freeze Dryer, UK). Dry mass was estimated to the nearest 0.00001 g. Subsequently, to establish fat content, the dried tissue was ground, weighed to the nearest 0.00001 g and packed into individual envelopes made of filter paper. All organs and the carcass of a given individual were put together into a Soxhlet extractor (Büchi extraction system B-811, Switzerland) where hot extraction of fat was conducted with petroleum ether (boiling range 40–60°C, Chempur, Poland). All organs were weighed again after fat extraction to estimate their fat content and to quantify total fat content of the whole individual.

The study was carried out under licence from the Local Ethical Committee on Animal Testing at Jagiellonian University (decision 95/2013).

Metabolic water and energy production

To estimate the overall energy and water budget of the experimental birds, we summed data on fat and protein content of all analysed organs for each individual (see Tables S1–S3). We calculated mean values for birds with access to food and water. Those means were subtracted from values obtained for individuals in the two fasting groups. The resulting estimates indicate use of endogenous resources by birds fasting with available water and fasting without water relative to birds having *ad libitum* access to food and water. We assumed total water production of 1.10 g H_2O g^{-1} of wet lipids and 0.82 g H_2O g^{-1} of wet protein and energy density of 37.6 kJ g^{-1} of wet lipid and 6.3 kJ g^{-1} of wet protein (Jenni-Eiermann and Jenni, 2012).

Metabolic rate

BMR was measured at +30°C, which lies in thermal neutral zone (Calder, 1964), in darkness in the α-phase (Aschoff and Pohl, 1970), as rates of oxygen consumption and CO_2 production (ml min^{-1}) using a 7-channel open-flow positive-pressure respirometric system with a S-3A/II O_2 analyser (AMETEK, Pittsburgh, PA, USA) and a CA2A CO_2 analyser (Sable Systems Inc., Las Vegas, NV, USA) (see Sadowska et al., 2015, for details). Respirometric chambers were rectangular 850 ml plastic containers.

Dried air was passed through the chambers at ca. 450 ml min^{-1} and the flow rate was regulated separately for each chamber by GFC-17 thermal mass-flow controllers (AALBORG, Orangeburg, NY, USA) calibrated against a LO 63/33 rotameter (Rota, Germany). The chambers were fitted with wire tops suspended 3 cm below the ceiling of the chamber (10 cm above the bottom) with the air inlet near the bottom and the outlet at the top of the chamber. Thus, the birds could not exhale air directly into the air outlet. Dried samples of air for the reference and sample channel were regulated sequentially through a V3 Intelligent Multiplexer (Sable Systems Inc.). The air stream was pre-dried with ND2 non-chemical dryer (Sable Systems Inc.) and dried by a magnesium perchlorate column before passing through the CO_2 and O_2 analysers. One sampling cycle for seven birds lasted 11.3 min, with the reference channel and the first measurement channel measured for 111 s, while the remaining five channels were active for 93 s, a length that ensured a complete washout of the system after switching channels. Mean values of analog outputs from the O_2 and CO_2 analysers were recorded once per second with a UE-9 AD interface (LabJack Corporation, Lakewood, CO, USA) using DAQ Factory acquisition system (Azotech, Ashlans, OR, USA). A total of 16 cycles was recorded. We used the last 20 s before switching channels to calculate the rate of O_2 uptake ($\dot{V}_{O_2}$). BMR was operationally defined as the mean of the two lowest recorded $\dot{V}_{O_2}$ from the last four cycles (the last 34 min), when all the birds were in a post-absorptive state (3 h after the last potential food intake in the case of control birds). Inactivity of each bird was confirmed for the respective metabolic rate measurements and the three preceding minutes by only accepting the typical background noise of the movement detector (MAD-1 gravimetric detectors, signal of 0–5 V range; Sable Systems, Inc.). For more details on the calculations, see Sadowska et al. (2015). All BMR values were obtained for birds at rest.

Use of ingestible water

For individuals that had access to water during the daytime, we quantified its use by weighing water dispensers before and after the period when the birds were allowed to drink. We controlled for evaporative water loss by quantification of evaporation in dispensers in empty cages. The data allowed comparison of water use between the control group and the group allowed access to water only. We found that birds in the control (food and water) group used on average 0.24 g of water per bird per hour, while birds that had only

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Table 1. The effects of food and water availability on body composition

	Group		Initial M_b				
	F_{df}	P	F_{df}	P	Control	Water only	No food or water
M_b at 08:00 h (g)	$0.12_{2,26}$	0.8884	$947.17_{1,26}$	<0.0001	14.29±0.06	14.259±0.06	14.269±0.06
M_b at 15:00 h (g)	$26.69_{2,26}$	<0.0001	$314.21_{1,26}$	<0.0001	14.44±0.10[a]	13.90±0.1[b]	13.39±0.10[c]
M_b at dissection (g)	$29.70_{2,26}$	<0.0001	$451.99_{1,26}$	<0.0001	13.94±0.08[a]	13.38±0.08[b]	13.10±0.08[c]
Water content (%)	$0.69_{2,27}$	0.5106			68.31±0.39	68.87±0.39	68.52±0.39
Lean mass (g)	$9.96_{2,26}$	0.0006	$157.14_{1,26}$	<0.0001	12.74±0.09[a]	12.23±0.09[b]	12.29±0.09[b]
Lean dry mass (g)	$5.33_{2,26}$	0.0115	$38.77_{1,26}$	<0.0001	3.03±0.04[a]	2.85±0.04[b]	2.87±0.04[b]
Total body fat (g)	$8.24_{2,26}$	0.0017	$35.38_{1,26}$	<0.0001	1.19±0.09[a]	1.14±0.09[a]	0.80±0.09[b]
% Body fat	$4.65_{2,27}$	0.0184			8.4±0.86[a]	8.29±0.86[a]	6.03±0.86[b]
BMR (ml O_2 min^{-1})	$3.63_{2,27}$	0.0400			0.65±0.02[a]	0.61±0.02[a,b]	0.58±0.02[b]
Mass-specific BMR (ml O_2 min^{-1} g^{-1})	$0.49_{2,27}$	0.6181			0.047±0.002	0.046±0.002	0.045±0.002

Birds were given access to food and water (control), or were deprived of food (water only) or of both food and water. Body mass (M_b) was measured in the morning (08:00h), at the end of the feeding regime (15:00 h) and at dissection. Water content is given as a percentage of lean carcass mass. BMR, basal metabolic rate. Mixed effect models were used, with experimental group as a fixed factor, initial body mass as a covariate (in relevant analyses) and day of dissection as a random effect (results not shown). Least square means±s.e.m. from the models are presented. Different letters indicate significant differences in pair-wise comparisons.

water used 0.4 g of water per bird per hour, yet the difference was not statistically significant (U=18, N=7, 8, P=0.27).

Statistical analyses

In all statistical models, group (*ad libitum* access to food and water, food deprivation with water, food deprivation without water) was a fixed factor and day of dissection (corresponding to one of the 7 days in which birds were recruited to the experimental groups) was a random effect. In all analyses of body parts, we used initial body mass as a covariate. This approach enabled investigation of differences in response variables while taking into account individual differences in body size before the experimental manipulations. In analyses of body composition expressed as percentages, body mass was not included. Metabolic rate was analysed at the level of the whole organism and also as a mass-specific value obtained by dividing BMR by body mass at the time of dissection. All response variables were checked for normality and they met that assumption.

We had no *a priori* predictions for the effect of sex on the measured traits, but we included this factor in our initial analyses. In all cases, the effect of sex and the sex-by-group interaction appeared non-significant. Thus, to increase statistical power of the analyses designed to compare the three experimental groups, our final tests did not account for the sex effect. All analyses were performed in SAS and graphs were prepared in Statistica.

RESULTS

Initial measurements

Birds assigned to the three experimental groups did not differ in tarsus length and initial body mass (see Materials and methods). All

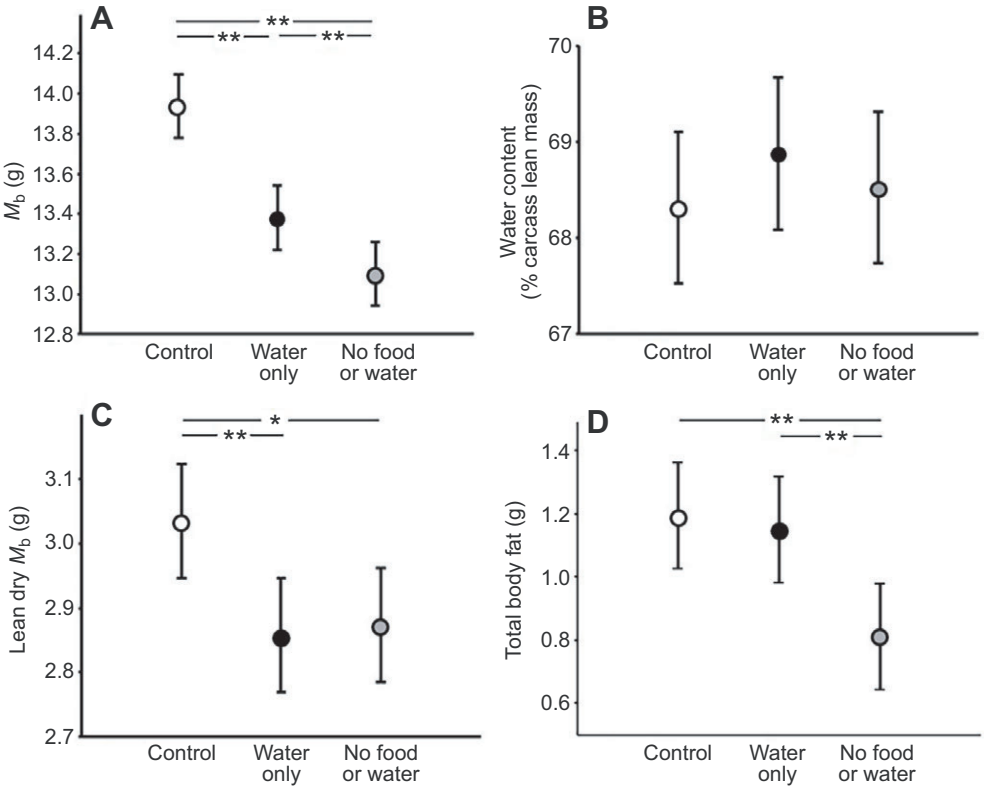

Fig. 1. Differences in body mass and composition in the three experimental groups at the time of dissection. Birds were given access to food and water (control), or were deprived of food (water only) or of both food and water. (A) Body mass (M_b), (B) body water content, (C) lean dry M_b and (D) total body fat. Least square means (LSM) and 95% confidence intervals from the models including initial M_b and day of dissection (see Table 1) are presented. Asterisks indicate significant pair-wise comparisons: *P<0.05, **P<0.01. Sample size was 12 in each group.

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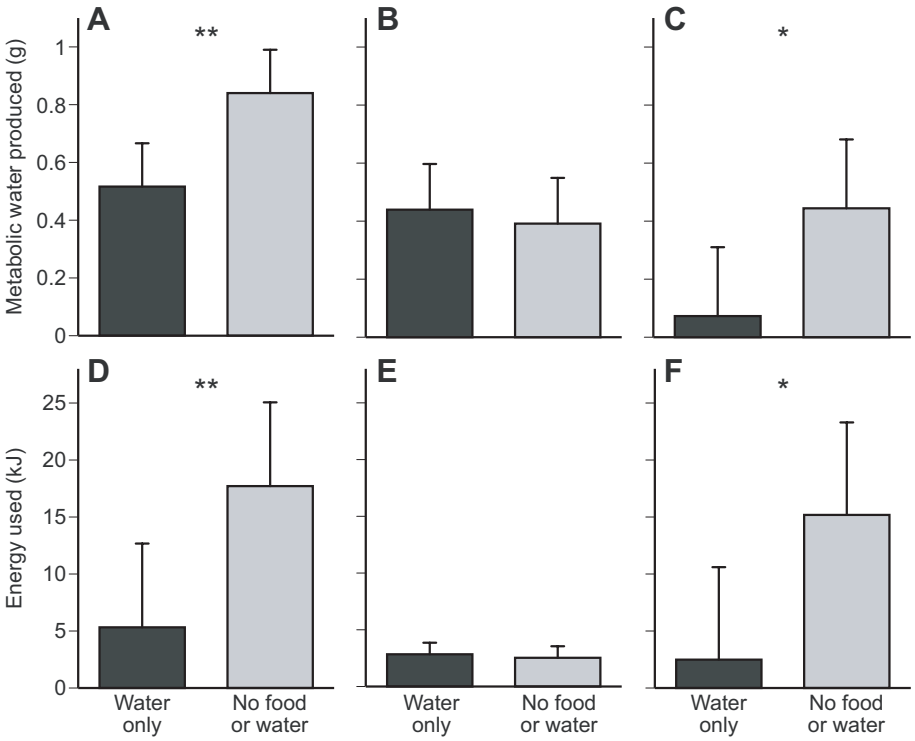

Fig. 2. Metabolic water and energy production in the two groups deprived of food. (A) Total gains in metabolic water from combined resources, (B) gains from wet protein and (C) gains from fat. (D) Total energy used, (E) energy from protein and (F) energy from fat. Estimates are based on the difference in body composition of the two experimental groups compared with birds with access to food and water. We assumed total water production of 1.10 g H_2O g^{-1} of wet lipids and 0.82 g H_2O g^{-1} of wet protein, and energy density of 37.6 kJ g^{-1} of wet lipid and 6.3 kJ g^{-1} of wet protein (Jenni-Eiermann and Jenni, 2012). LSM and 95% confidence intervals from the models including initial M_b and day of dissection are presented (see Results, 'Metabolic water and energy production'). Asterisks indicate significant differences between the groups: *P<0.05, **P<0.01. Sample size was 12 in each group.

the birds lost a comparable body mass during overnight fasting, resulting in a lack of difference in mean body mass in the morning (at 08:00 h; Table 1).

Body mass and body composition following the feeding regimes

The feeding regimes significantly affected body mass both at the end of the feeding regime (at 15:00 h; Table 1) and at the time of dissection (Table 1, Fig. 1A). At the time of dissection, overall hydration of the birds expressed as a percentage of carcass lean mass (Table 1, Fig. 1B) did not differ among the groups. The three groups differed in terms of total fat content, percentage of body fat, lean body mass and lean dry body mass of the individuals (Table 1, Fig. 1). *Post hoc* analyses revealed that, at the time of dissection, body mass of the food-deprived birds with access to water was intermediate, significantly lower than that of birds with access to food and water (control, P<0.0001) and greater than that of food-and-water-deprived birds (P=0.0174), which were the lightest (Fig. 1A). Birds fasting with access to water had the lowest lean body mass, significantly different from the control group (P=0.0004), but not different from the food-and-water-deprived birds (P=0.65). The latter were significantly different only from controls (P=0.0013). Lean dry body mass showed a similar pattern, with control birds being significantly different from birds fasting with water (P=0.0065) and birds fasting without water (P=0.0126; Fig. 1C). Birds fasting with water maintained their total body fat at similar levels to the control birds (P=0.65), while individuals without access to food and water showed a significantly lower fat mass than birds with access to both resources (P=0.0010) and birds fasting without water (P=0.0030; Fig. 1D). Differences in percentage body fat followed the same pattern (Table 1).

Metabolic water and energy production

Catabolised fat was on average 0.047 g in birds fasting with water and 0.385 g in birds deprived of food and water. Catabolised lean tissue was on average 0.453 g in birds fasting with water and 0.511 g in birds fasting without water.

Water availability for fasting birds had a profound effect on their metabolic water production and mobilised body resources. Birds that were deprived of both food and water produced in total 1.6 times more metabolic water than birds deprived of food but with access to water (experimental group: $F_{1,15}$=10.58, P=0.0053; body mass: $F_{1,15}$=274.88, P<0.0001; Fig. 2A) and consequently used significantly more energy for that purpose (experimental group: $F_{1,15}$=9.33, P=0.008; body mass: $F_{1,15}$=36.66, P<0.0001; Fig. 2D). This was not accomplished by differential catabolism of lean wet matter, which was found not to differ between the two groups. Specifically, there were no differences in water and energy (both analyses; experimental group: $F_{1,15}$=0.35, P=0.5632; body mass: $F_{1,15}$=137.72, P<0.0001; Fig. 2B,E) released as a result of catabolism of lean wet mass. However, fat catabolism yielded 6 times higher metabolic water and energy production in birds fasting without water compared with birds that were fasted but had access to water (both analyses; experimental group: $F_{1,15}$=8.49, P=0.0107; body mass: $F_{1,15}$=17.19, P=0.0009; Fig. 2C,F).

BMR of the whole organism differed among the groups and was significantly lower in food-and-water deprived birds compared with control birds (P=0.0132; Table 1). Food-deprived birds with access to water had intermediate BMR that was not significantly different from the two other groups. There were no differences in mass-specific BMR among the groups (Table 1).

DISCUSSION

Our study is the first to investigate the effects of water availability on body composition and energy budget in fasting individuals that were not forced to fly or thermoregulate. Contrary to the hypothesised breakdown of protein to serve the purpose of liberating water (Klaassen, 1996; Bauchinger and Biebach, 1998; Jenni and Jenni-Eiermann, 1998), lack of drinking water did not

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cause elevated catabolism of protein of the fasting birds; in fact, lean mass declined similarly in the two fasting groups. However, lack of water speeded up body mass loss of the fasting birds as a result of elevated fat catabolism; overall fat level was 42% lower than that of birds with access to drinking water (Fig. 1). Thus, water balance of birds fasting without water seemed to be maintained mainly by elevated fat catabolism, which generated 6 times more metabolic water compared with birds with access to water (Fig. 2).

In birds fasting without access to water, the above-mentioned fat catabolism yielded 0.444 g of metabolic water (Fig. 2). To produce the same amount of water from protein, these birds would have to catabolise 0.54 g of lean wet mass, which would double its actual catabolism. In fact, 0.54 g of lean wet tissue would amount to, for example, over twice the remaining liver mass (see Table S1), or more than 3 times the remaining heart mass, or about one-third of the remaining flight muscle mass or more than 5 times the remaining kidney mass. Although protein catabolism would probably be spread over different organs, this exemplifies that catabolism of such an amount of protein would severely constrain tissue function, not only during fasting but also when food intake is re-established.

If protein had been differentially catabolised between the two fasting groups, we would have expected to also detect a difference in BMR. Neither whole-animal nor mass-specific BMR differed between the fasting groups (Table 1), and, therefore, the metabolic rate measurements were consistent with the results for gross body composition. Adipose tissue is largely inert in its contribution to metabolic rate and, thus, the large difference in fat tissue may not be reflected in the BMR measurements. BMR reflects momentary energy expenditure, while energy production is calculated from changes in body composition (Fig. 2) and represents a cumulative value over the entire period of differential availability of resources. The latter indicates significantly higher overall energy expenditure of the birds fasting without access to water. We have two potential explanations for this effect. The first explanation is that birds deprived of food and water increased activity to generate metabolic water from fat. The second explanation is that fat catabolism is accompanied by heat production and thus generates additional costs (in terms of water and energy) related to heat dissipation, which is considered an important process, often constraining physiological performance (e.g. Speakman and Król, 2010). Such an effect would be a self-feeding cycle. However, as we did not monitor activity of the birds during the time when their feeding regimes were differentiated, the above explanations remain speculative, opening a new avenue of research.

Metabolic water production may be the only way to maintain water balance in a dry habitat. It is essential when food does not provide enough water, or in extreme situations when feeding is not possible, for instance during sandstorms or heat waves, which can cause mass mortality of birds (McKechnie and Wolf, 2010). Species inhabiting arid zones manage to maintain water balance even though they are not necessarily more flexible than species from mesic habitats (Tieleman et al., 2003). Zebra finches are naturally familiar with dry environmental conditions and they are well-adapted to cope with a long-term water shortage (Cade et al., 1965; Zann, 1996). At the same time, they are highly sensitive to water deprivation even in the presence of food. For instance, male singing behaviour is reduced after water deprivation and is rapidly restored when water becomes available (Cynx, 2001; Rashotte et al., 2001). It has been shown that water-deprived zebra finches deposit more lipids in the intercellular spaces of the skin than individuals with drinking water available (Menon et al., 1989). Deposition of lipids in the skin reduces cutaneous water loss (Menon et al., 1989) and could be interpreted as an acclimation response to conserve water. However, it is not known whether such a strategy may also serve to build up fuel for metabolic water production. Our finding of a fat-saving effect of water may indicate that birds deprived of food and water would be less likely to survive because of smaller fat reserves (or a higher rate of fat catabolism) than birds deprived of food but with access to drinking water.

Our study suggests that, in the absence of food, elevated consumption of water may contribute to the physiological pattern of fasting. Potentially, the availability of water made the birds sated for longer. Thus, we will compare our findings with what is known about the effect of water in animals that have access to food and discuss the possibility that water postponed the transition from phase I to phase II of fasting.

The finding that water availability helped to save fat reserves resembles the effects of the availability of water on birds in an anabolic state, such as during a migratory stopover. In such situations, drinking water helps to boost body mass increase and fat accumulation in blackcaps, *Sylvia atricapilla* (Sapir et al., 2004; Tsurim et al., 2008). However, the effect is driven by the improvement of food processing and water balance rather than by changes in food metabolisability (Tsurim et al., 2008). Furthermore, physiological effects of drinking water include faster regeneration of the digestive track (Mizrahy et al., 2011), which would not take place in food-deprived birds. In the current study, birds were in a catabolic state, as they were fasted for 24 h. However, our results might indicate that birds with and without access to water could have been in different phases of fasting.

Generally, animals deprived of food undergo three consecutive phases of fasting (Bar and Volkoff, 2012; Cherel and Le Maho, 1985; Cherel et al., 1988; Jenni-Eiermann and Jenni, 2012). Phase I is the time of adjusting to long-term fasting; it starts with a high rate of carbohydrate and protein catabolism (causing high body mass loss) and ends with low rates of body mass loss. In phase II, most of the energy is delivered from lipids and the rate of body mass loss is minimised. The length of each phase is said to depend on the initial fat stores of the individual, but it is not known what triggers the transition from phase I to phase II. In turn, phase III starts when fat reserves reach threshold adiposity and protein catabolism increases dramatically (Jenni-Eiermann and Jenni, 2012). In small passerines, threshold adiposity is a fat level of 10–5% of total dry mass (Schwilch et al., 2002). Given that in our study, the level of fat as a percentage of total dry mass was in the range 12–17%, we can assume that none of the birds reached phase III of fasting.

The phases of fasting were described for animals deprived of food, but with access to water (Cherel and Le Maho, 1985; Cherel et al., 1988; Klaassen and Biebach, 1994). Those studies have (unstated) assumptions that the timing and order of utilisation of endogenous resources is governed by the need to preserve proteinaceous tissue and the need for energy. Our results suggest that transitions between the phases may also depend on the need for metabolic water. We found that the two groups deprived of food showed a similar fast decline of lean dry mass (Fig. 1C), which is typical for phase I of fasting. The group that was deprived of water experienced a significant decline of fat stores (Fig. 1D), which could suggest that those birds went into phase II faster than birds deprived only of food. To verify whether a lack of water indeed shortens phase I of fasting, some additional experiments with manipulation of water availability within the metabolic chamber, restriction of physical activity and constant analyses of respiratory quotient would be required. One would also have to demonstrate that in a small passerine, phase I of fasting might be extended throughout the night time.

Conclusions

Our study offers a novel hypothesis of fat-for-water by clearly demonstrating that the lack of ingestible water greatly increases catabolism of fat. Fat is the major source of energy and its breakdown also produces metabolic water. It has previously been assumed that animals at rest use protein (instead of fat) catabolism to sustain water balance. Here, we show that fat is a primary source of metabolic water production under routine energy requirements of fasting, in the absence of physiological conditions that require elevated energy expenditure. We suggest that maintenance of water budget by increased protein breakdown could possibly occur if fat stores are depleted or if fat catabolism is already elevated to its upper limits as a result of high energy demands or if the rate of water loss exceeds the potential rate of water production from fat. Follow-up studies should examine whether the mechanism behind our finding is related to increased activity of the birds deprived of food and water.

Acknowledgements

Mariusz Borowski and Jonatan Kozłowski provided technical help during the study. Two anonymous reviewers gave insightful comments on the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

All authors took part in collecting the data and interpreting the results. J.R. carried out the statistical analyses and lead writing of the manuscript; E.T.S. carried out BMR measurements and tissue analyses; M.C. helped draft the manuscript; U.B. conceived, designed and coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

Funding

The study was financed by the Jagiellonian University (Uniwersytet Jagielloński w Krakowie) statutory funds: DS 757 (K/ZDS/004151).

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.138966.supplemental

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