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Energetics and metabolite profiles during early flight in American robins (*Turdus Migratorius*)

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Abstract Although birds use fat as the primary fuel for migratory flights, carbohydrate and protein catabolism could be significant in the early stages of flight while pathways of fatty acid transport and oxidation are induced. The fuel mixture of long distance migrant birds can also be affected by the rate of water loss, where birds catabolize more protein to increase endogenous water production under dehydrating flight conditions. Despite many studies investigating flight metabolism, few have focused on the metabolic response to flight during the switchover to fat catabolism in migrants, and none have examined the effect of ambient conditions on fuel selection during early flight. We investigated the effect of water loss on the metabolic response to short duration flight in the American robin (*Turdus migratorius*). Birds were flown in a climatic wind tunnel and changes in body composition and plasma metabolites were measured. As flight duration increased, there was a gradual switchover from carbohydrate and protein catabolism to fat catabolism. Plasma metabolite profiles indicate that the mobilization of fat occurred within 20 min of initiating flight. Plasma glucose decreased and uric acid increased with flight duration. Ambient humidity did not affect fuel mixture. Thus, it seems that the utilization of fat may be delayed as migrants initiate flight. Short-hop migrants may exploit high rates of endogenous water production resulting from carbohydrate and protein catabolism early in flight to offset high water loss associated with low humidity. Rapid catabolism of lean body components at the start of a flight also reduces mass quickly, and may reduce energy costs.

Keywords Plasma metabolites · Flight · Bird migration · Flight energetics · Quantitative magnetic resonance

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Introduction

Flight is the most energetically demanding form of locomotion (Schmidt-Nielson 1972), and migratory birds are unique among vertebrates in their ability to fuel this high-intensity exercise for long durations with fat (Jenni and Jenni-Eiermann 1998; Guglielmo 2010). The use of fat in flight is evidenced by the fact that migratory birds increase adiposity coinciding with migratory periods (i.e., spring and fall; Jehl Jr 1997); these fat stores are depleted after long flights in the wild, and replenished quickly during stopover (Battley et al. 2000; Guglielmo et al. 2005; Guglielmo et al. 2011). Other metrics of fat use in flight, such as high plasma free fatty acid concentrations, respiratory quotients near 0.7, upregulation of enzymes associated with fat catabolism, and reductions in fat mass measured with quantitative magnetic resonance also confirm the high rates of fat catabolism in flight (Jenni-Eiermann and Jenni 1991; Jenni-Eiermann et al. 2002; Landys et al. 2005; Rothe et al. 1987; McFarlan et al. 2009; Lundgren 1988; Gerson and Guglielmo 2011a). In addition, ruffs (*Philomachus pugnax*), a long distance migrant shorebird, use fat oxidation to fuel a majority of energy demand in many activity states (rest, shivering and

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running) regardless of rate of energy expenditure or duration (Vaillancourt et al. 2005).

Carbohydrate and protein catabolism are important during the early phase of flight, before the physiological and biochemical pathways for fat transport and oxidation are induced. For example, it can take up to 30 min for fatty acid transporters to be translocated from the cytosol to the membrane in rat muscle (Bonen et al. 2000; Pelsers et al. 2008). Presumably a similar process occurs in birds, but this has not been investigated directly. In pigeons (*Columba livia*), however, fat use does not stabilize until after 1–2 h of flight, indicating a gradual switchover to fat oxidation (George and John 1993; Rothe et al. 1987). This switchover may be much faster in migratory birds due to their enhanced ability to mobilize and catabolize fat (Jenni-Eiermann et al. 2002; Guglielmo 2010). The high relative contribution to energy from fat oxidation is well established in birds in steady prolonged flight, but the rate of switchover to fat oxidation during early flight has not been thoroughly investigated in migratory passerines.

In addition to provisioning energy during the early stages of flight, the catabolism of carbohydrates and protein also yields about five-times more metabolic and bound water than the catabolism of fat (Jenni and Jenni-Eiermann 1998). It has been hypothesized that preferential oxidation of carbohydrate and protein could be used to offset high rates of water loss in flight (Carmi et al. 1995; Klaassen 1995, 1996). Recently, we showed that birds flown for long durations (up to 5 h) under high evaporative water loss conditions do, in fact, increase the rate of protein catabolism (Gerson and Guglielmo 2011a). Thus, it is now apparent that the rate of protein catabolism varies in response to ambient conditions during flight in birds, and fuel mixture is adjusted to meet both the energy and water requirements. Although many studies have investigated fuel mixture in flight or during exercise in birds, the important contribution of protein has often been ignored (for reviews of fuel mixture in flight see Jenni and Jenni-Eiermann 1998; McWilliams et al. 2004; Price 2010; Weber 2011).

Plasma metabolite analysis can provide insight into exercise metabolism of birds (Bordel and Haase 1993; George and John 1993; Gerson and Guglielmo 2011a; Guglielmo et al. 2001; Jenni-Eiermann et al. 2002). High plasma concentrations of ß-OH-butyrate, non-esterified fatty acids (NEFA), and glycerol are indicative of high rates of fat mobilization during exercise (Jenni-Eiermann et al. 2002; Vaillancourt et al. 2005). It has also been proposed that triglycerides are used by small passerines to increase the concentration of circulating fatty acids in the plasma, once albumin has been saturated with NEFA (Jenni-Eiermann and Jenni 1992). This alternative fat transport pathway has been documented only in wild small passerine birds caught mid-flight during their annual migration (Jenni-Eiermann and Jenni 1991; Jenni-Eiermann and Jenni 1992). Uric acid is increased in circulation when rates of protein catabolism are high (Bordel and Haase 1993; Gerson and Guglielmo 2011a; Gerson and Guglielmo 2011b). Thus, examination of changes in plasma metabolites can provide information on the metabolic response to flight of various durations, and the effects of in-flight humidity on fuel mixture.

The goal of this study was to investigate fuel use and the metabolic response to flight during the switchover period in a passerine migrant, the American robin (*Turdus migratorius).* We tested three hypotheses: (1) protein and carbohydrate catabolism would be greatest early in a flight, and would decline as flight duration increased, (2) low humidity in flight would prolong the switchover period, increasing the use of protein and/or carbohydrates in flight, and (3) plasma triglyceride concentration would increase during flight as a pathway of fatty acid delivery. We used quantitative magnetic resonance body composition analysis (QMR) to measure changes in fat and lean (protein) masses during flight in a wind tunnel, and plasma metabolite concentrations were determined before and after flight to examine the metabolic response to short duration flight under high and low humidity conditions.

Materials and methods

Animal care

Twenty-four American robins (*Turdus migratorius*) were caught near London, Ontario, Canada (43°04′28″ N, 81°20′14″ W) from July through September 2009. Eight of these birds were brought into captivity as nestlings early in summer and hand-raised, which seemed to have little benefit for flight propensity, or calmness in captivity. Four birds were caught at Long Point, Ontario, Canada as adults during fall migration, and the rest were caught as hatch year birds in the summer. Birds were maintained in smooth-walled aviaries (3.7 m × 2.4 m × 3.1 m) on natural light cycle until March 2010. At this time they were switched to long light cycle (16L:8D) to induce migratory disposition, as indicated by mass gain. Birds were fed ground Mazuri® Small Bird Diet (Purina Mills LLC) supplemented with berries and mealworms. 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. 2010-216). Birds were captured under a permit from the Canadian Wildlife Service (CA-0256).

Wind tunnel

Birds were flown in a wind tunnel that was specifically designed for bird flight and allows the researcher to independently control humidity and temperature within the range of 0–90 % RH at temperatures up to 30 °C, while also controlling wind speed between 0 and 20 m s^{-1}. For a technical description of the wind tunnel see (Gerson and Guglielmo 2011a).

Experimental design and changes in body composition

Birds were initially trained in the wind tunnel for a period of 2 weeks starting March 1, 2010 to identify birds with a natural inclination to fly in the wind tunnel, and to try to increase flight durations of birds that were initially less inclined to fly. Birds were trained to sit on a perch held by the researcher. Once perched, birds were moved into the test section of the wind tunnel, which was set at a wind speed of 3–5 m s^{-1}. Wind speed was gradually increased until birds started to take voluntary "test" flights, returning to the perch in between flights. As birds voluntarily increased the duration of these test flights, the perch was removed to encourage sustained flight. The perch was placed back in the tunnel once the birds started looking for a place to land. With this strategy, we trained the birds not to land on the bottom of the test section of the tunnel. Total training time did not exceed 30 min in a day. Birds were trained individually or in pairs. Over the course of a few training sessions, flight durations increased in some birds. Once training was completed, birds were given 1–5 days to recover before the start of the experiment to replenish fat reserves. Of the 24 birds in captivity all were tested in the wind tunnel but only 12 showed inclination to fly and were thus used for flight experiments. Birds were housed in pairs in rolling cages (1.2 m × 0.7 m × 1.8 m) during the experiment. This facilitated catching the birds for weighing and blood sampling. Food was removed 3 h prior to flight to ensure birds were post-absorptive and all fuels catabolized in flight were endogenous. Flights commenced either at 13:00, or at 18:00 each day. Despite this period of food restriction, birds did sometimes produce droppings in flight, which could not be collected and weighed without disturbing the flying bird. Immediately prior to the flight, birds were weighed, and scanned using QMR (Guglielmo et al. 2011), and were hand released into the wind stream. Birds flew at a wind speed of 12 m s^{-1}, at 18 °C, and absolute humidity was either 12 g H_2O m^{-3} (80 % RH; LEWL) or 2 g H_2O m^{-3} (13 % RH; HEWL). In order to determine the metabolic response to humidity, we attempted to fly each individual bird once under each humidity condition, where the initial condition was determined randomly. All flights by an individual bird commenced at the same time of day. Two days prior to a scheduled flight, birds were food restricted for 3 h, exactly as on the day of the flight, and blood samples were taken by brachial puncture. Thus, these blood samples were taken at the same time of day as the scheduled commencement of the flight, and the metabolite levels should approximate those of birds on flight days. We attempted to collect a maximum of two capillary tubes ($\sim$75 µl each), but some birds lost slightly more before bleeding was stopped. After the completion of a flight, birds were scanned a second time using QMR, weighed, and bled within 5 min. Birds were given 6 days to recover before the repeated experimental flight under the other humidity treatment. We attempted to match the initial flight duration during the second flight, but few birds successfully completed a flight of matching duration. Flight costs were calculated from changes in QMR fat and lean mass assuming 39.6 kJ g^{-1} for fat, and 5.3 kJ g^{-1} for lean mass (Jenni and Jenni-Eiermann 1998).

Plasma metabolite analysis

All blood samples were centrifuged at 13,000×*g* (IEC MicroCL 17, Thermo-scientific hematocrit centrifuge), hematocrit was determined and the plasma fraction was drawn off and stored at −80° C until analysis. All plasma metabolite assays were performed using standard clear 96 well plates and a microplate spectrophotometer (Biotec Powerwave X340). Uric acid was analyzed in undiluted plasma. For all other metabolites, plasma was diluted threefold in 0.9 % saline. Glycerol, and triglyceride concentrations were measured by endpoint assay and ß-OH-butyrate was measured by kinetic assay as in Guglielmo et al. (2002a) and Guglielmo et al. (2005). Uric acid was determined using a commercially available kit as in Gerson and Guglielmo (2011a). Plasma NEFA, glucose and phospholipid (PL) concentrations were determined by endpoint assay using commercially available kits (NEFA-HR(2), Autokit Glucose, and Phospholipid C; Wako Diagnostics) as in Guglielmo et al. (2005).

Statistical analysis

Since the dataset contained repeated measures in an unbalanced design, general linear mixed models were used with individual as a random factor for all analysis (Zuur et al. 2009). Models were determined using backward stepwise selection where non-significant ($P > 0.05$) terms were dropped sequentially until only significant terms remained, and only these terms are reported in the results. All analyses initially included initial body mass and flight duration as covariates. Statistical comparisons between pre- and post-flight concentrations of plasma metabolites

were made using paired *t* tests. Humidity treatments were pooled if no significant differences existed, as model selection progressed. Of the 10 completed flights, we were able to acquire seven blood samples post-flight. Due to the low sample size, general linear mixed models would sometimes not converge. In these cases, due to the low number of repeated measures on a single individual, we used general linear models. This occurred with change in lean mass and all of the plasma metabolites except NEFA.

Results

Changes in body composition and flight energetics

During the experimental timeframe, 10 flights were completed by 6 birds where body composition and plasma samples were collected. Of these flights, 4 were completed under the HEWL conditions, and 6 under the LEWL conditions. Only 3 birds completed a flight under both conditions, but flight durations were not matched. Birds flew an average of 37.23 min (range 17.88–54.46 min), and there was no significant difference in flight duration between humidity treatments ($F_{1,4.98} = 2.02$, $P = 0.215$). Mean pre-flight mass was 74.59 ± 1.15 g, whereas mean post-flight mass was 73.13 ± 1.341 g. Longer flight durations resulted in greater reduction in mass ($F_{1,5.031} = 59.893$, $P = 0.001$), fat mass ($F_{1,7.712} = 112.631$, $P < 0.001$), and lean mass ($F_{1,8} = 10.605$, $P = 0.012$; Fig. 1), but no significant effect of humidity treatment was evident (mass: $F_{1,3.751} = 0.064$, $P = 0.813$; fat mass: $F_{1,3.698} = 0.560$, $P = 0.499$; lean mass: $F_{1,7} = 0.296$, $P = 0.603$).

Birds catabolized a fuel mixture consisting primarily of fat, but a considerable proportion of the energy required for flight was derived from lean mass (Fig. 2). The relative proportion of energy from fat increased with flight duration ($F_{1,7.712} = 112.631$, $P < 0.001$, Fig. 2a), while the relative proportion of energy from lean decreased ($F_{1,8} = 10.605$, $P = 0.012$; Fig. 2a). Overall mean flight costs were determined to be 14.90 W and, unexpectedly, there was a significant increase in estimated flight costs with flight duration ($F_{1,7.221} = 12.337$, $P = 0.009$; Fig. 2b). Although the proportion of energy from lean mass was 20.9 ± 2.96 %, catabolism of lean mass accounted for 52.36 ± 0.07 % of the mass loss. There was no effect of pre-flight mass on flight costs ($F_{1,5.334} = 0.55$, $P = 0.823$), and thus mass was not included as a covariate in the model testing the effect of flight duration on flight costs.

Plasma metabolites

There was a linear increase in post-flight uric acid levels with flight duration ($F_{1,6} = 16.204$, $P = 0.007$; Fig. 3).

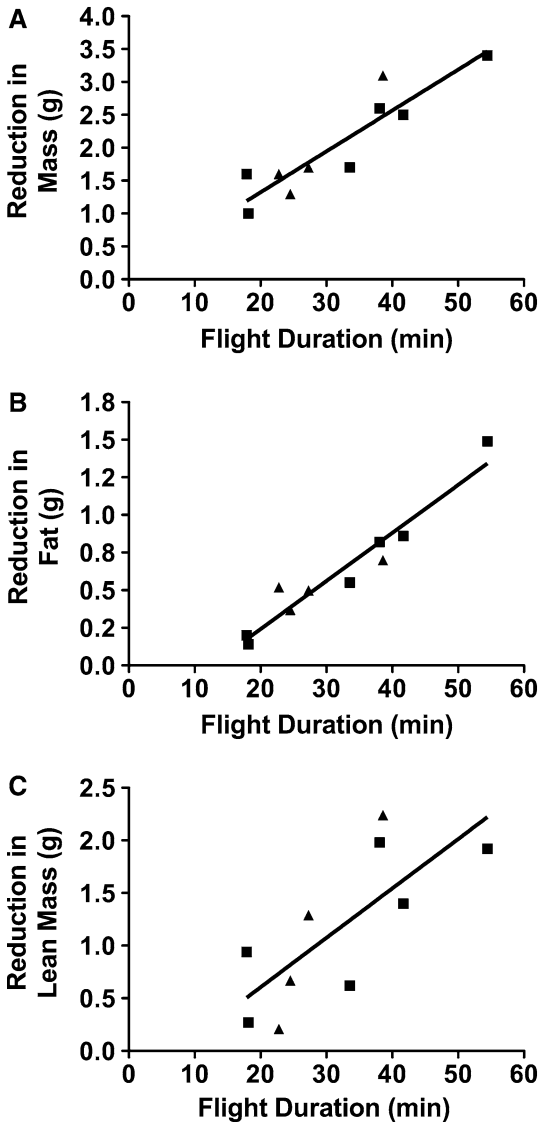

Fig. 1 The reduction in mass, fat mass, and lean mass in American robins as a function of flight duration. Flight resulted in significant reductions in mass, slope −0.061 ± 0.008 g min^{-1} (**a**), fat mass −0.033 ± 0.003 g min^{-1} (**b**), and lean mass effect size: −0.0469 ± 0.144 g min^{-1} (**c**). There was no effect of humidity treatment on the rate of body composition change. See text for statistical details. HEWL *squares*, LEWL *triangles*

NEFA tended to decrease slightly with flight duration, but this trend was not significant ($F_{1,3.485} = 3.632$, $P = 0.140$). ß-OH-Butyrate and hematocrit remained constant throughout the flight (ß-OH-Butyrate: $F_{1,5} = 2.186$, $P = 0.199$; Hematocrit: $F_{1,5} = 1.378$, $P = 0.293$). No other metabolites showed obvious relationships with flight duration (triglycerides: $F_{1,5} = 1.378$, $P = 0.293$; phospholipid: $F_{1,5} = 0.393$, $P = 0.558$; glucose: $F_{1,5} = 0.569$, $P = 0.485$; glycerol: $F_{1,5} = 0.543$, $P = 0.494$).

Comparisons between pre- and post-flight metabolite levels using paired *t* tests indicated significant increases in

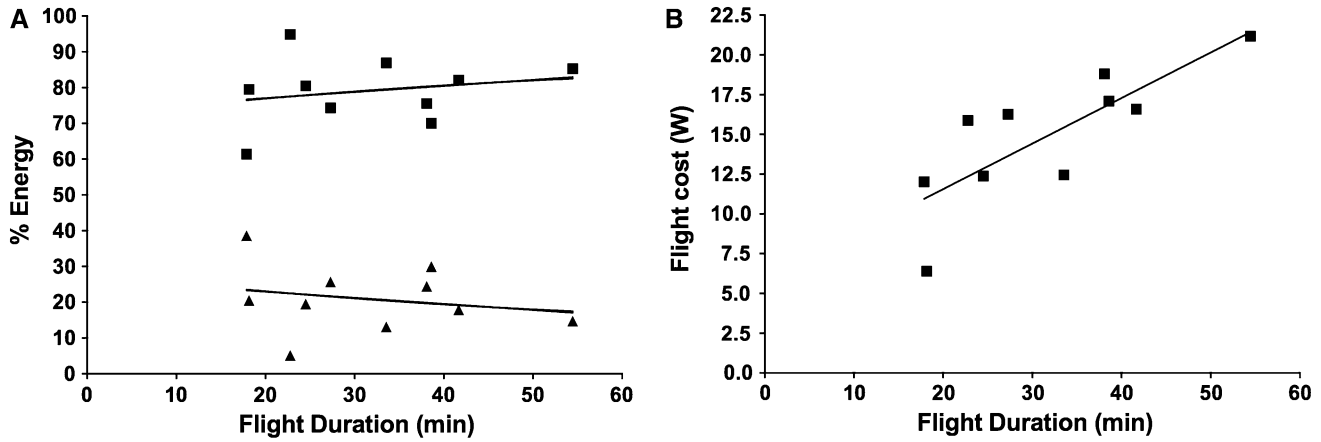

Fig. 2 The relative contribution of fat and lean mass to total flight costs in American robins. **a** As flight duration increased, there was a significant reduction in the relative proportion of energy derived from lean mass (*triangles*), and a corresponding increase in the relative proportion of energy from fat (*squares*). **b** As flight progressed flight costs increased. See text for further explanation and statistical details

glycerol ($t = 5.707$, DF = 6, $P = 0.001$), ß-OH-butyrate ($t = 5.843$, $DF = 6$, $P = 0.001$), NEFA ($t = 23.193$, $DF = 6$, $P < 0.001$), and uric acid ($t = 1.535$, $DF = 7$, $P = 0.05$) after flight. Plasma triglycerides did not change significantly during flight, but tended to decrease rather than increase ($t = 0.746$, $DF = 6$, $P = 0.484$). Phospholipids ($t = 4.113$, $DF = 5$, $P = 0.009$) and glucose ($t = 9.898$, $DF = 6$, $P < 0.001$) were significantly reduced post-flight (Fig. 4).

Discussion

Although metabolic switchover during exercise has been thoroughly documented in many species of mammals (Weber 2011), there are few studies directly investigating this phenomenon during endurance flight in birds (Schwilch et al. 1996, Rothe et al. 1987). Birds may be fundamentally different in terms of their fuel selection strategy during long duration, high-intensity flights, where extra-muscular lipids are the primary fuel source. A reduction in respiratory quotient as flight duration increases has been demonstrated in pigeons (Rothe et al. 1987), but here we use changes in body composition and plasma metabolite profiling to characterize the switchover period. Within the first 30 min of flight, 70 % of energy was derived from fat, and the relative contribution from fat increased gradually as flight duration increased. This indicates a rapid switchover to fat oxidation in these birds, but maximal rates of fat oxidation were not achieved until later in the flight.

Robins lost a surprising amount of mass even during short flights, some more than 3.0 g in 55 min, yet flight costs were not reduced in birds that experienced greater mass loss, as was expected. Differences in flight costs between humidity treatments likely would not be detected within such a short duration flight. However, rapid mass loss early in a flight could, theoretically, reduce overall flight costs for longer duration flights. Flight costs, as determined by changes in body composition, increased with flight duration. Calculating flight costs for short duration flights has long been difficult in the study of flight physiology and many techniques have been used (Engel et al. 2006; Ward et al. 2004). The use of the doubly labeled water method is accurate for long flights; while mask respirometry is commonly used for shorter flights even though the drag produced by a mask and tether may affect flight costs. Heart rate as a proxy for metabolic rate and heat transfer modeling have also been used to calculate flight costs during short duration flights, but require specialized equipment and validation within a species (Ward et al. 2004). The use of QMR to calculate flight costs has not been directly validated against more established methods, yet it does provide comparable flight costs for long duration flights (up to 5 h) in small songbirds (Gerson and Guglielmo 2011a). The energy costs of flight that we calculated from QMR data are similar to those of comparably sized European starlings (*Sturnus vulgaris*); 11.85 W for birds flying at 12 m s^{-1} by Ward et al. (2004). Using the equation presented by Masman and Klaassen (1987) flight cost for wind tunnel flight using the mean mass of birds used in the current study was 13.93 W. Based on these two comparisons it seems that QMR provides a reasonable measurement of true flight costs. However, the costs of the short duration flights in the present study may be misrepresented by QMR because energy sources relied upon early in flight may simply not be reflected in changes in body composition. Catabolism of plasma or even

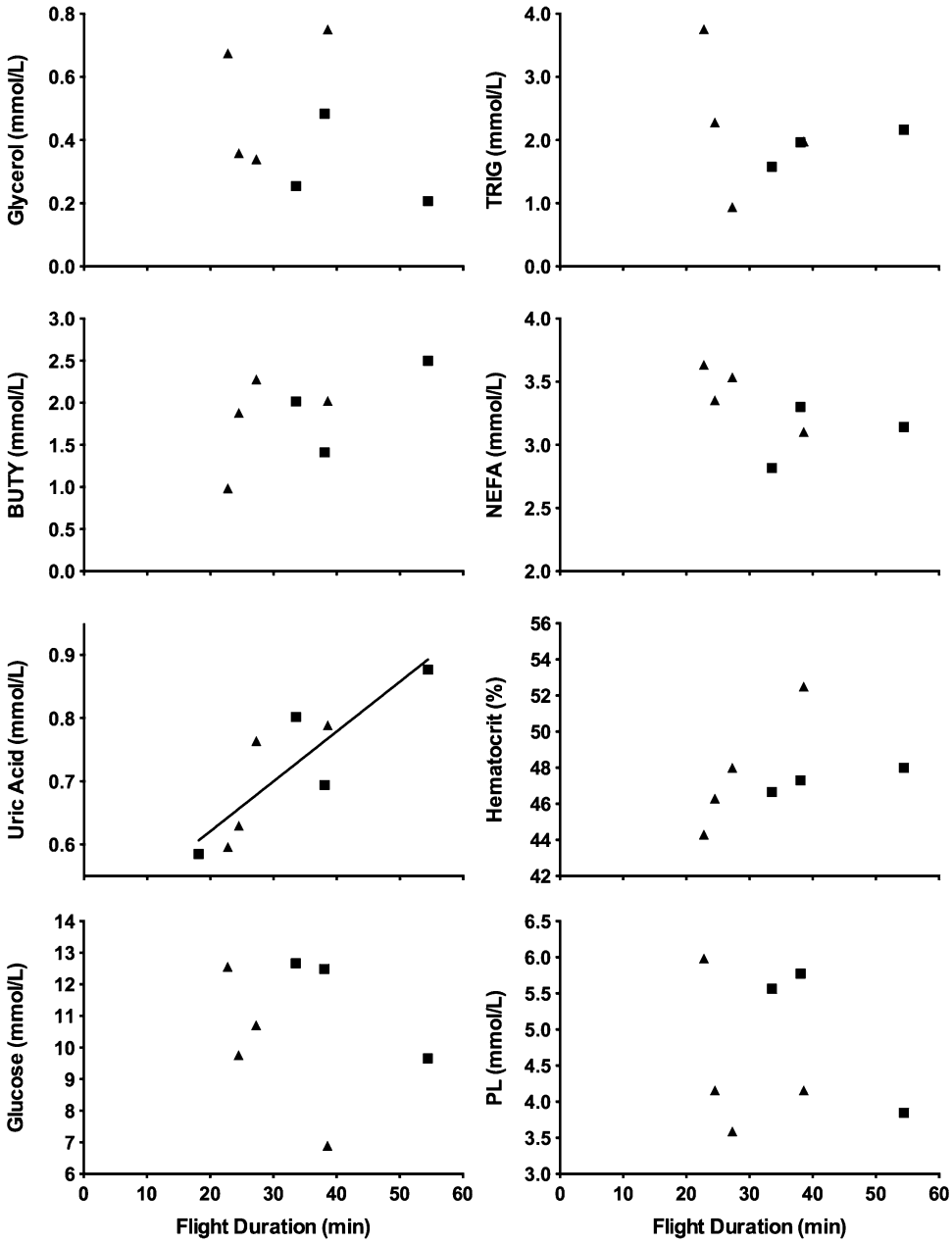

Fig. 3 Plasma metabolite concentrations in response to flight duration in American robins. There was no effect of humidity on any of the plasma metabolites, but treatments are shown. LEWL *Triangles*, HEWL *squares.* Only uric acid increased with flight duration as indicated by the regression line. See text for statistical details

intramuscular glucose or other metabolites may not be detected with QMR. For these reasons we interpret this increase in flight costs with flight duration cautiously.

It has been suggested that experimental flights in wind tunnels may be more costly than free flight (Masman and Klaassen 1987). Engel et al. (2006) address this topic in detail but due to the low turbulence in the wind tunnel used for this study this potential difference in flight costs has likely been minimized.

In a previous study of Swainson's thrushes (*Catharus ustulatus*), we found that the rate of lean mass catabolism increased when birds flew for 1–5 h under the same low humidity conditions as used in the current experiment (Gerson and Guglielmo 2011a). The present results show that early in flight there is no detectable effect of humidity on the rate of lean mass catabolism in American robins. During short flights the large contribution of lean mass to fuel during the switchover period produces substantial quantities of water, irrespective of the environmental conditions. Thus, these birds may in fact be in water surplus early in flight and likely rely on excretory water losses as a means of reducing mass, a strategy that would aid in extending flight range or reduce flight costs due to lower average mass over the course of a flight.

Interestingly, there was a slight decrease in plasma triglyceride concentration post-flight. It has previously been

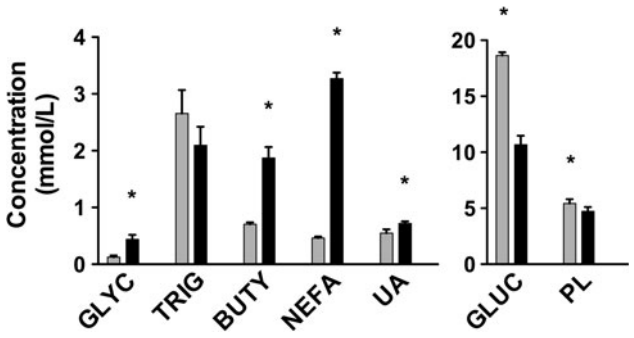

Fig. 4 Plasma concentrations of metabolites measured before (*grey bars*) and after (*black bars*) flight. *GLYC* Glycerol, *TRIG* triglyceride, *BUTY* ß-OH-butyrate, *NEFA* non-esterified fatty acids, *UA* uric acid, *GLUC* glucose, *PL* phospholipids. *Asterisk* indicates a significant difference between pre- and post-flight levels within a metabolite ($P < 0.05$)

suggested that during migratory flight small passerine birds use very low-density lipoproteins high in triglycerides to transport fatty acids in high concentration through the blood, and that this augments the NEFA-bound-to-albumin transport pathway (Jenni-Eiermann and Jenni 1992; Price 2010). The evidence for such a strategy has been mixed, where larger birds and birds flown in wind tunnels show reduced or stable plasma triglyceride in response to flight, and wild-caught small migrants show increased triglyceride (Jenni-Eiermann and Jenni 1992; Jenni-Eiermann et al. 2002). The current study, using relatively large American robins ($\sim$70 g) provides more evidence that triglycerides, and therefore the lipoprotein transport pathway, are not up-regulated during flight in larger passerine birds. However, due to the large quantity of lipid contained in circulating triglycerides, triglycerides may still have a role in fat transport during long distance migration (Landys et al. 2005). The levels of NEFA are very high in American robins post-flight, which suggests that transport by NEFA is likely sufficient to fuel flight for short durations.

With the exception of uric acid, there was no effect of flight duration on post-flight concentrations of any of the measured plasma metabolites. The increase in uric acid up to 1 h is similar to what has been observed early in flight in red knots (*Calidris canutus*) flown in a wind tunnel for up to 12 h (Jenni-Eiermann et al. 2002) and in homing pigeons free flying for up to 5 h (Schwilch et al. 1996). In red knots, uric acid concentrations reached a plateau after the first hour, and then remained stable for the remainder of the flight, whereas pigeons showed a linear increase in uric acid with flight duration, similar to what was observed in the current study (Schwilch et al. 1996). ß-OH-Butyrate increased quickly upon the initiation of flight, reaching a plateau after about 20 min, which was maintained for the duration of the flight. This is in agreement with studies on metabolite profiles of other migrant birds flown in a wind tunnel, although our study provides better resolution of the rapid response of ß-OH-Butyrate to exercise in birds (Jenni-Eiermann et al. 2002). Surprisingly, NEFA showed a decreasing trend with flight duration, but the magnitude of the in-flight concentration was substantially higher than resting values, reaching levels as high as 3.6 mmol L^{-1}. This value is higher than what has been previously documented for long distance migrants. Jenni-Eiermann et al. (2002) flew red knots in a wind tunnel for up to 12 h, but plasma NEFA concentrations were maintained around 1.0 mmol L^{-1} in most birds. Similarly, birds caught in flight during migration had plasma NEFA concentrations between 1.0 and 2.0 mmol L^{-1} (Jenni-Eiermann and Jenni 1992). This may indicate a very high initial lipolytic rate in robins that overshoots demand early on, before steady state is achieved. Alternatively, this could be a product of the time between the end of the flight and when the blood sample is collected, as NEFA concentrations could peak due to the sudden stop of exercise (Jenni-Eiermann and Jenni 2001; Romijn et al. 1993). Birds were weighed and scanned in the QMR before blood was taken, but this entire process was completed within 5 min of ending the flight.

When compared to pre-flight levels there were significant increases in glycerol, NEFA, and ß-OH-butyrate, all of which are indicative of high rates of fat catabolism, and of uric acid. Glucose was reduced in response to flight indicating carbohydrate metabolism initially, but due to the high levels of NEFA and ß-OH-butyrate, reduced reliance on glucose is likely for the duration of flight. Previous studies have shown no change in blood glucose with flight (Jenni-Eiermann et al. 2002). Blood glucose levels may recover once steady state flight is reached, but the apparent inability to maintain blood glucose could also indicate a greater reliance on glucose in flight for these birds. If this reliance on glucose is not reduced as flight progresses, it could result in greater lean mass catabolism to sustain gluconeogenesis.

The metabolic response early in flight may be substantially different between long- and short-distance migrants, and further investigation of this possibility may provide insight into the metabolic adaptations that allow extreme long duration flight in migratory birds. We would also like to emphasize that considering both the water and energy budgets during this period of flight is critical to fully understand how these animals have evolved to cope with varied environmental conditions during flight.

Our study shows the value of QMR as a tool for the rapid and accurate measurement of changes in body composition of animals, as well as the determination of flight energetics. It appears that costs of short flights may not be accurately represented by changes in body composition, illustrating a limitation to the use of this technique. It seems that despite the heavy pressures to maximize fat

utilization for long migratory flights, there may be some benefit to using low energy density fuels early in flight to maximize the rate of mass loss. Such a strategy would provide long-term energy savings, especially for larger birds, or long-distance migrants, but the use of such a strategy needs to be more thoroughly investigated. The plasma metabolite profiles of American robins in flight fit closely with previous published values, yet this study provides increased resolution to the metabolic response in the early stages of flight.

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