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Carbohydrate absorption by blackcap warblers (*Sylvia atricapilla*) changes during migratory refuelling stopovers

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SUMMARY

Passerine birds migrating long distances arrive at stopover sites to refuel having lost as much as 50% of their initial body mass (m_b), including significant losses to digestive organs that may serve as a reservoir of protein catabolised for fuel during flight. Birds newly arrived at a stopover show slow or no m_b gain during the initial 2–3 days of a stopover, which suggests that energy assimilation may be limited by reduced digestive organs. Measurements of migrants and captive birds subjected to simulated migratory fasts have shown reductions in intestine mass, morphological changes to the mucosal epithelium, and reductions in food intake and assimilation rate upon initial refeeding. We found that blackcaps (*Sylvia atricapilla*, Linnaeus) newly arrived at a migratory stopover after crossing the Sahara and Sinai deserts had significantly increased paracellular nutrient absorption (non-carrier mediated uptake occurring across tight junctions between enterocytes) that may provide partial compensation for reduced digestive capacity resulting from changes to intestinal tissues. Indeed, newly arrived birds also had a slightly reduced capacity for absorption of a glucose analogue (3-*O*-methyl-D-glucose) transported simultaneously by both carrier-mediated and non-mediated mechanisms. Increased paracellular absorption coupled with extended digesta retention time may thus allow migratory blackcaps to maintain high digestive efficiency during initial stages of refuelling while digestive organs are rebuilt.

Key words: migration, refuelling, carbohydrate absorption, paracellular absorption, blackcap, *Sylvia atricapilla*.

INTRODUCTION

Every boreal autumn, most bird species that breed in northern latitudes migrate south to wintering grounds and return the following spring. Birds that are long-distance migrants break their flight with periodic stopovers, when they rest and refuel (Biebach et al., 1986; Biebach et al., 2000; Gannes, 2002; Lavee and Safriel, 1989; Lavee et al., 1991). The flight of passerine birds, which often includes individual legs of up to several hundred kilometres, is fuelled mainly by fat (~85%) and to a lesser extent by protein (~15%) (Klaassen et al., 2000). Birds preparing for migration store fat mainly in the peritoneal cavity and subcutaneously (Blem, 1976; Gill, 2007), but there is no specific site or organ for protein storage. Recent evidence suggests that birds use organs that are inactive during flight (kidneys, spleen and alimentary organs), as well as skeletal and heart muscles, as a reservoir of protein that can be catabolised to power flight (reviewed by Bauchinger et al., 2005). During a trans-Saharan flight, some birds lose as much as 50% of their initial body mass (m_b) (Bauchinger et al., 2005; Biebach, 1998; Pennycuick and Battley, 2003). Loss of mass from the organs of the gastrointestinal tract (GIT) is accompanied by significant changes in their morphology and function (Karasov et al., 2004). In particular, intestinal villi of captive blackcap warblers (*Sylvia atricapilla*, Linnaeus) subjected to simulated migratory food restriction or fasting were significantly shorter and had significant disintegration at their tips compared with those in fed blackcaps. Fasted and food-restricted birds had lower food intake and assimilation rates than birds that had recovered by refeeding for 2–3 days (Karasov and Pinshow, 2000; Karasov et al., 2004), indicating that the morphological changes observed do indeed translate into reduced capacity for nutrient assimilation.

m_b in blackcaps increases slowly during the first 2–3 days of a migratory stopover but increases rapidly on subsequent days (Bauchinger et al., 2009; Gannes, 2002; Karasov and Pinshow, 2000), suggesting that the initial reductions in GIT mass and function limit energy assimilation. In addition to potentially causing reduced mediated uptake of nutrients, the migration-related changes to the intestine may also increase permeability of the intestinal epithelium (Karasov et al., 2004), facilitating greater paracellular absorption (i.e. non-carrier-mediated uptake that occurs across tight junctions between adjacent enterocytes). Paracellular uptake can be an important avenue for the absorption of sugars and other water-soluble nutrients in small birds (Afik et al., 1997; Caviedes-Vidal et al., 2007; Chediack et al., 2001; Karasov and Cork, 1994; Levey and Cipollini, 1996; McWhorter et al., 2006). In this light, small passerine migrants might benefit from increased paracellular absorption, especially when mediated absorption of nutrients is limited by migration-related reductions in amount of intestine.

With the above in mind, we predicted that blackcaps that have crossed the Sahara and Sinai deserts in spring while heading north would have higher paracellular (passive, non-mediated) and lower carrier-mediated absorption of carbohydrates upon arrival at a stopover site than they would after several days of refeeding. To test this we applied a standard pharmacokinetic method to measure the absorption of sugars by newly arrived and refed blackcaps in

Eilat, Israel. We measured paracellular absorption with three different sized inert probes that are absorbed by only non-mediated mechanisms [L-arabinose (molecular mass=150 Da), L-rhamnose (164 Da) and cellobiose (342 Da)]. Because paracellular absorption depends on molecule size, we predicted that fractional absorption of these probe compounds would be in the rank order arabinose > rhamnose ⩾ cellobiose (Chediack et al., 2003). As an index to total glucose absorption, which occurs by non-mediated pathways as well as by carrier-mediated pathways, we also measured the absorption of 3-*O*-methyl-D-glucose (a non-metabolisable D-glucose analogue; hereafter 3OMD-glucose). Analysis of probes absorbed by both mediated and non-mediated pathways provides a more complete understanding of mechanisms of absorption by the birds during their migratory stopover.

MATERIALS AND METHODS

Experimental animals

Blackcaps were captured in Eilat, southern Israel (29°33′N, 34°57′E) from 3 April to 22 April 2005 in Rybachy-type traps (Payevsky, 2000) at the International Birding and Research Centre, Eilat (IBRCE). The birds were ringed (banded) with standard aluminum rings (size S, Tel-Aviv University, Israel) and divided into two groups for experiments on the absorption of nutrients in the intestine: new arrivals and refed birds. Because trapping at the IBRCE went on for several weeks prior to our project, we considered a bird to be newly arrived to Eilat if it was captured in the morning and bore no ring. In addition, because birds were continually captured and ringed at the IBRCE, we were also able to judge waves of new arrivals by the frequency at which un-ringed birds were captured. During the spring migration, blackcaps typically arrive in Israel in pulses (Izhaki and Maitav, 1998; Shirihai, 1996). At the beginning of a migratory pulse, the proportion of un-ringed blackcaps is high, but as they remain at the IBRCE to refuel over the subsequent days, the proportion of birds caught without rings declines sharply (R. Yosef, unpublished observations). Thus, we only allocated birds to the new arrivals group on mornings when most of the captured blackcaps were ringless. Gannes found that blackcaps that were captured repeatedly over several days at a stopover site (i.e. those that were probably recently arrived) were significantly lighter upon initial capture than birds that were never recaptured (i.e. those that had probably already refed at the site before initial capture) (Gannes, 2002). Therefore, as a final step to ensure that the birds were in the initial phases of tissue rebuilding, we only used birds with m_b upon capture <14.5 g for the new arrivals treatment (Fig. 1). This was less than the mean m_b of all blackcaps captured at the IBRCE during the course of the present study [16.8±1.7 g (mean±s.d.), N=774]. We measured uptake in birds assigned to the new arrivals treatment immediately after capture.

Birds that were allocated to the refeeding group were placed in a large outdoor aviary (16 m × 10 m × 2 m) that was covered with 50% shade netting and contained several shrubs for additional cover. Because blackcaps are more frugivorous during migration than when breeding or wintering (Cramp, 1992; Shirihai et al., 2001), we supplied fresh fruit (primarily watermelon, but also other melons, apples and tomatoes) *ad libitum*, and the birds were able to supplement their diet with free-ranging arthropods that were attracted to the fruit. Although by our selection criteria these birds could have already been refeeding, we held and fed them for several more days to ensure that their GITs had fully recovered function. We weighed the blackcaps daily to ±0.1 g, and most maintained or lost mass over the first 2–5 days and then maintained or gained mass (Fig. 1). This timeframe is comparable with that seen in free-ranging blackcaps

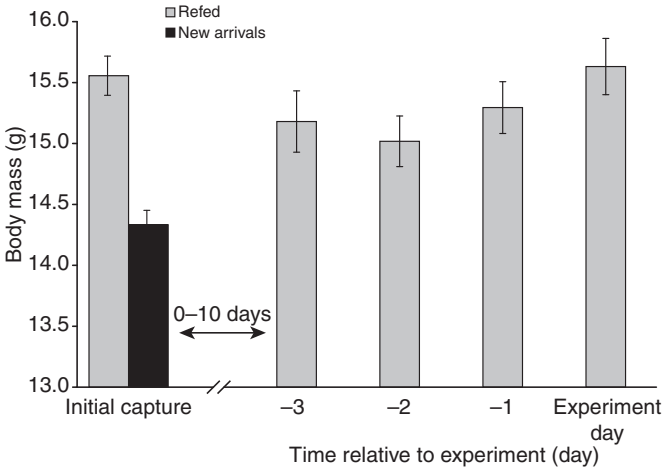

Fig. 1. Mean body mass (±s.e.m.) of migratory blackcaps allocated to the new arrival (N=37) and refed (N=36) treatments upon initial capture, and during refeeding in the refed treatment. Birds allocated to the new arrival treatment had body masses upon capture <14.5 g and were captured on mornings when most blackcaps were un-ringed. Birds allocated to the refed treatment initially maintained or lost mass for the first several days of refeeding (range: 1–10 days), and were removed for uptake measurements when they had gained mass for two consecutive days.

(Gannes, 2002). Blackcaps refeeding after a fast are able to restore GIT mass and function in two days (Karasov et al., 2004), so we judged the birds in the aviary to have restored GIT function when they gained mass for two consecutive days. Most birds increased in mass for two consecutive days after 3–5 days but some required up to 10 days to do this. Once the birds met this criterion, we removed them from the aviary for uptake experiments.

Paracellular uptake experiments

Birds from both treatment groups were randomly assigned to receive a solution containing carbohydrate probes, either by oral gavage (N=21 new arrival, N=18 refed) or injection into the pectoral muscle (N=16 new arrival, N=18 refed). This solution included three carbohydrates that are only passively absorbed by paracellular pathways (L-arabinose, molecular mass=150 Da; L-rhamnose, 164 Da; and cellobiose, 342 Da), and one that is absorbed *via* both mediated and paracellular mechanisms (3OMD-glucose, 194 Da). This combination of probes allowed us to determine the relative importance of paracellular uptake, as well as the effects of molecular size on paracellular nutrient absorption (Chediack et al., 2003; Tracy et al., 2007). The probe solution contained 70 mmol l^{-1} L-arabinose, 70 mmol l^{-1} L-rhamnose, 50 mmol l^{-1} cellobiose, and 100 mmol l^{-1} 3OMD-glucose, and was brought to 350 mmol l^{-1} (isosmotic with bird blood) with 30 mmol l^{-1} of NaCl. Birds in the gavage and injection treatments were dosed 2% and 0.2% of their m_b (measured as mass of probe solution per mass of bird), respectively.

Blood samples (~50 µl) were drawn from the brachial vein for a background measurement before administration of the probe and then at 7, 15, 25, 45, 90 and 150 min afterwards. Between samplings, birds were kept in individual cages covered with dark cloth. The standard use of this pharmacokinetic procedure requires sampling from the same individuals for all blood samples and for each individual to receive both gavage and injection treatments (Chang and Karasov, 2004; McWhorter and Karasov, 2007; Tracy et al., 2007). However, because of their small m_b, and consequently small blood volume, we were only able to take two blood samples from

any individual blackcap. Therefore, we randomly assigned two sampling times to each bird and used 73 birds to create a composite time series with data from multiple independent blood samples at each sampling time. Thus, we obtained five or six samples per sampling time for each treatment of gavage or injection, and newly arrived or refed birds. After the second sample, birds were offered water and fresh watermelon and were released on site at the IBRCE.

Blood samples were immediately centrifuged for 2 min at 10,000 ***g*** to separate plasma from cells. Plasma mass was determined to ±0.1 mg, and then the samples were stored frozen at –20°C until further processing. Probe concentrations in plasma were determined by high performance liquid chromatography (HPLC) as in Tracy et al. (Tracy et al., 2007).

Pharmacokinetic calculation of absorption

The mean dose and m_b-corrected plasma concentrations of each probe, C [ng probe (mg plasma)$^{-1}$ (g dose)$^{-1}$ (g m_b)$^{-1}$], were plotted as a function of sample time, t (min). The absorbed amounts of the various probes were calculated from areas under the post-absorption and post-injection plasma curves (AUC=area under the curve of plasma probe concentration *vs* time). This simple method does not require assumptions about pool number or size, or kinetics (Welling, 1986). Fractional absorption (f), also called bioavailability, was calculated as:

$$f = \mathrm{AUC}_{\mathrm{oral}} / \mathrm{AUC}_{\mathrm{injection}}. \quad (1)$$

Following standard pharmacokinetic procedures (Welling, 1986), the area from t=0 to t=x minutes (when the final blood sample was taken) was calculated using the trapezoidal rule. The area from t=x to t=∞ was calculated as:

$$\mathrm{AUC}^{x\rightarrow\infty} = (C \text{ at } t{=}x) / K_{\mathrm{el}}, \quad (2)$$

where K_{el} (min^{-1}) is the elimination rate constant for removal of the probe from plasma, estimated by non-linear fitting of post-injection data (Tracy et al., 2007), pooled for all birds from the peak concentration in plasma to the final blood sampling time. The total $\mathrm{AUC}^{0\rightarrow\infty}$ was obtained by summing the two areas.

Statistical analyses

Because we could not take a complete series of blood samples from any individual bird, the mean of probe concentrations from five or six different birds (Fig. 2) was used at each sampling time to calculate AUCs needed to calculate f. This resulted in single f values for each probe in each treatment (i.e. newly arrived and refed birds). To make statistical comparisons, we used a jackknife resampling procedure to generate pseudovalues (Quinn and Keough, 2002). In this procedure, values are recalculated up to N times with one data point removed and then replaced before the next resample. To apply this method to the present data, we removed one data point at all seven blood sampling time points for both gavage and injection treatments simultaneously, recalculated mean plasma probe concentrations and then replaced these data points before removing the next set. This resulted in six pseudovalues for each treatment, and thus a total sample size of N=7 for statistical analyses. Note that when doing statistical analyses on such data, one must assume that pseudovalues are independent of each other, while in reality they are not (Crowley, 1992; Robertson, 1991).

Numerical results are given as means ± s.e.m. (N=number of animals unless otherwise indicated). f values for probes were arcsine-square root transformed prior to statistical comparisons (Sokal and Rohlf, 1995). Analysis of variance (ANOVA) was used on pseudovalues to test for differences in f among treatments and probes, with Tukey HSD *post-hoc* contrasts as appropriate. Significance was accepted at $\alpha \leq 0.05$. We also considered $\alpha < 0.1$ to indicate a significant trend because using composite retention curves constructed from data taken from several birds (as opposed to curves created from serial blood samples from a single bird) results in a loss of statistical power.

RESULTS

Mean plasma probe concentrations after injection generally peaked at the first blood sampling time of 7 min, except in the cases of L-arabinose and cellobiose in newly arrived blackcaps where it peaked at 15 min (Fig. 2). In gavage trials, mean plasma probe concentrations generally peaked at 15 min except for cellobiose in refed blackcaps, where probe concentration peaked at 7 min. This pattern was expected because both digesta transit from stomach to intestine and the absorption process cause delays in the probe reaching plasma, compared with the administration by injection.

There was a trend (i.e. $0.05<P<0.1$) for lower f of 3OMD-glucose in the newly arrived birds (0.625±0.016) compared with the refed blackcaps (0.719±0.045; $F_{1,12}$=3.54, P=0.084; Fig. 3A). By contrast, and as predicted, f of paracellular probes was significantly higher in newly arrived blackcaps than in refed blackcaps ($F_{1,36}$=10.31, P=0.0028). There were significant differences in f among the probes ($F_{2,36}$=232.76, P<0.0001, all probes significantly different by Tukey HSD), and a significant interaction between probe and treatment ($F_{2,36}$=19.58, P<0.0001; Fig. 3B). f of cellobiose was significantly greater in newly arrived (0.244±0.018) than in refed birds (0.107±0.01; $F_{1,12}$=50.86, P<0.0001). There was a trend for higher fractional absorption of L-arabinose in newly arrived (0.515±0.033) than in refed birds (0.418±0.031; $F_{1,12}$=4.56, P=0.0539). f of L-rhamnose, however, was significantly lower in newly arrived (0.582±0.011) than in refed birds (0.678±0.017; $F_{1,12}$=21.49, P=0.0006).

f of the paracellular probes declined significantly with increasing probe molecular mass in new arrival samples ($F_{2,12}$=113.15, P<0.0001). With combined data for both treatments, this same pattern held ($F_{2,26}$=103.84, P<0.0001). Considering just the data from refed birds, there was also a significant effect of probe size ($F_{2,12}$=183.50, P<0.0001); however, the pattern of decline with probe size was less clear (i.e. L-rhamnose f >L-arabinose f).

DISCUSSION

Migratory blackcaps newly arrived at our study site showed evidence of both decreased capacity for carrier-mediated glucose absorption and increased paracellular (non-mediated) absorption of small, water-soluble carbohydrate compounds, relative to birds that had refed. Our results from wild migrants corroborate the reductions in digestive performance observed in captive blackcaps subjected to simulated migratory fasting (Karasov et al., 2004). Given these observations, two important questions come to mind: (1) what mechanism(s) explain the decrease in mediated absorption and increase in paracellular absorption, and (2) what impacts do these changes in intestinal structure and function have on digestive performance and ecology of birds during migratory stopovers?

Karasov et al. simulated migration conditions in blackcaps by subjecting captive birds to periods of fasting and food restriction (Karasov et al., 2004). These authors observed a substantial reduction in mass of GIT organs of fasted and food-restricted blackcaps compared with fed birds, accompanied by a deterioration of the small intestine mucosal epithelium and a decrease in villus length. Presumably, similar changes to the GIT occurred in birds in the present study [see Karasov and Pinshow (Karasov and Pinshow,

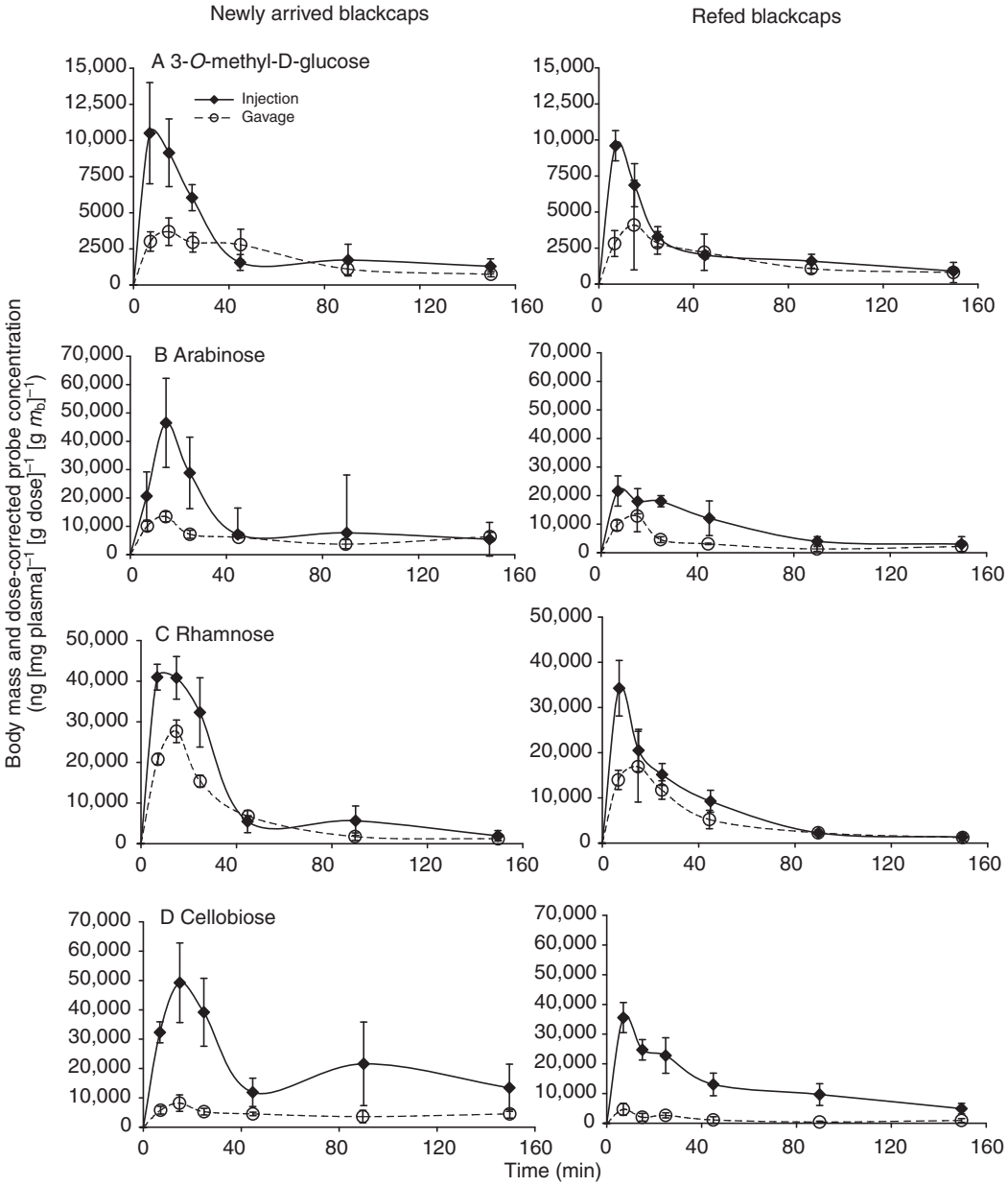

Fig. 2. Mean dose and body mass-corrected concentrations of probes in blackcap plasma (±s.e.m., *N*=5 or 6 at each value) after injection (filled diamonds) and oral gavage (unfilled circles) in newly arrived (left column) and refed birds (right column). 3-*O*-methyl-D-glucose, absorbed by both mediated and non-mediated mechanisms, is shown in row A, and the paracellular carbohydrate probes, absorbed only by non-mediated mechanisms, are shown in rows B–D.

1998) for data on gut morphology in wild migrants]. Mediated nutrient absorption is primarily associated with enterocytes located in villi, so the trend (P<0.1) of reduced 3OMD-glucose bioavailability observed in newly arrived migrant blackcaps in the present study is consistent with observed changes in gut morphology (Karasov et al., 2004). In studies with captive birds, refeeding for two days resulted in returns of epithelial structure, villus length, food intake and nutrient assimilation rates to pre-fast levels (Karasov and Pinshow, 2000; Karasov et al., 2004). This is consistent with greater 3OMD-glucose bioavailability in refed birds observed in the present study. The correlation between length of intestinal villi and capacity for mediated nutrient absorption provides a likely explanation for observations regarding 3OMD-glucose, but what of the paracellular probes absorbed *via* non-mediated pathways?

It is not clear from our data whether the increased absorption of paracellular probes in newly arrived birds resulted from an active (‘adaptive’) modulation of epithelial permeability, increased digesta retention time, or was simply a consequence of morphological changes to the intestinal epithelium (e.g. disintegration of villus tips) associated with migration (Karasov et al., 2004). All of these mechanisms seem plausible. Recent studies, using uniform methods, have shown that non-migrating, small, flying vertebrates rely more on paracellular absorption than do non-flying mammals, allowing them to maintain comparably high digestive assimilation efficiencies in spite of relatively shorter digesta retention times, generally smaller guts and lower absorptive surface area (Caviedes-Vidal et al., 2007). The mechanisms by which paracellular absorption may be modulated are poorly understood (see Anderson and van Itallie,

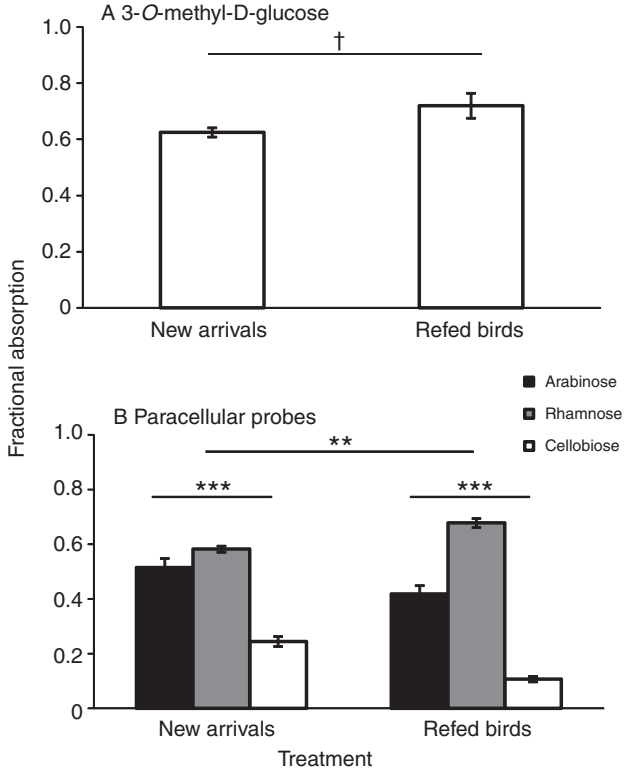

Fig. 3. Fractional absorption, or systemic bioavailability, of (A) 3-*O*-methyl-D-glucose and (B) the paracellular carbohydrate probes in new arrivals and refed birds. 3-*O*-methyl-D-glucose fractional absorption (the sum of mediated and non-mediated absorption) showed a significantly increasing trend between newly arrived and refed birds. Paracellular probe fractional absorption was significantly lower in the refed treatment; absorption was significantly different among all probes and generally decreased with increasing molecular mass (L-arabinose=150 Da, L-Rhamnose=164 Da, cellobiose=342 Da). † significant trend at $P<0.1$, **significant at $P\leq0.001$ and ***significant at $P\leq0.0001$. Fractional absorption values were calculated from composite curves based on samples from 37 newly arrived and 36 refed birds (see text for detail).

1995; Ballard et al., 1995; Fasano, 2008), but the observation that the presence of nutrients in the intestinal lumen of non-migrating passerine birds increases paracellular nutrient absorption (Chediack et al., 2003) suggests that modulation occurs under some circumstances. Recent measurements in nectar-feeding birds have shown that paracellular f increases with increasing sugar concentration in the diet (McWhorter et al., 2006; Napier et al., 2008). In these studies it is not possible to discern whether there was modulation of epithelial permeability or whether the increased paracellular absorption observed was a consequence of increased digesta retention time, or both.

Digesta retention time may be modulated to maintain digestive efficiency (Sibly, 1981), and increased paracellular absorption may be in some part simply a consequence of increased contact time between digesta and absorptive surfaces. In nectar-feeding birds, higher dietary sugar concentrations are associated with lower food intake rates, and longer digesta processing times (López-Calleja et al., 1997) and increased paracellular absorption (Napier et al., 2008). Interestingly, Bauchinger et al. have shown that digesta mean retention time in blackcaps newly arrived at migratory stopovers is significantly longer than in refed birds (Bauchinger et al., 2009). This allowed birds in their study to maintain high digestive efficiency throughout the refuelling period. Extended mean retention time may also partly explain the observation of increased bioavailability of paracellular probes in the present study. Migrating passerines at the beginning of a refuelling stopover would have both high epithelial permeability and reduced capacity for mediated transport. Longer mean retention times would thus maximise both paracellular nutrient absorption and mediated nutrient transport for refuelling birds.

Migratory birds exhibit remarkable phenotypic flexibility in alimentary organ size throughout their annual cycles (Hume and Biebach, 1996; Karasov and Pinshow, 1998; Karasov et al., 2004; Klaassen and Biebach, 1994; Piersma, 1998; Piersma and Gill, 1998). Reductions in digestive organ size during migration offers the dual benefits of reduced mass of tissue and digesta to be carried in flight and reduced resting metabolic rate (Battley et al., 2000), but may act as a primary physiological limitation to rate of assimilation and body mass gain during stopovers (Gannes, 2002; Karasov et al., 2004). The increased paracellular (non-mediated) uptake of water-soluble nutrients that we observed in newly arrived blackcaps may provide some compensation for putative reductions in mediated nutrient absorption capacity. Furthermore, this compensation may be supplemented by (and/or a consequence of) the longer digesta retention times that occur in blackcap warblers newly arrived at migratory stopover sites (Bauchinger et al., 2009). The biphasic increase in m_b in refeeding migratory birds reflects an initial slower period of rebuilding of lean tissue mass, followed by relatively more rapid increases in fat mass (Carpenter et al., 1993; Karasov and Pinshow, 1998; Wojciechowski et al., 2005), strongly suggesting that the GIT must be rebuilt before rapid fat accumulation can occur. This digestive limitation has important ecological implications; delays during stopover refuelling decrease a species' overall migration speed, increasing the chances of failure to reach breeding areas and decreasing the time available for reproduction (Alerstam and Lindström, 1990). Importantly, our previous understanding of the dynamics of alimentary organ condition and function during migratory stopovers has come primarily from simulations of migratory fasts (and subsequent refeeding), using captive birds (Karasov and Pinshow, 1998; Karasov and Pinshow, 2000; Karasov et al., 2004) (but see Gannes, 2002). However, the present study used wild-caught migrants in a direct test of the hypotheses stemming from prior work on captives. The results of these different studies concur to give a consistent picture of the dynamics of digestive function during migratory stopovers, despite the differences in techniques.

From an evolutionary perspective, high intestinal permeability entails both costs and benefits. On the one hand, Pappeheimer suggested that paracellular absorption may be selectively advantageous because it requires little energy (Pappeheimer, 1993). Absorption increases in a linear fashion with nutrient concentration (i.e. capacity does not saturate as in carrier-mediated systems), so absorptive capacity is automatically matched to nutrient load (McWhorter, 2005). This may provide a digestive advantage for long-distance migrants arriving at stopovers with atrophied digestive organs. On the other hand, increases in epithelial permeability that permit increased paracellular absorption may also result in increased absorption of toxins from plant or animal material in the intestinal lumen (Diamond, 1991), a cost in terms of fitness. Greater systemic exposure to hydrosoluble toxins could significantly increase metabolic costs for detoxification, and thus could be an important selective force limiting the breadth of the dietary niche in refuelling migratory passerine birds.

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REFERENCES

Afik, D., McWilliams, S. R. and Karasov, W. H. (1997). A test for passive absorption of glucose in yellow-rumped warblers and its ecological implications. *Physiol. Zool.* **70**, 370-377.

Alerstam, T. and Lindström, A. (1990). Optimal bird migration: the relative importance of time, energy, and safety. In *Bird Migration: Physiol. Ecol.* (ed. E. Gwinner), pp. 331-351. Berlin: Springer.

Anderson, J. M. and van Itallie, C. M. (1995). Tight junctions and the molecular basis for regulation of paracellular permeability. *Am. J. Physiol.* **269**, G467-G475.

Ballard, S. T., Hunter, J. H. and Taylor, A. E. (1995). Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. *Ann. Revi. Nutrition* **15**, 35-55.

Battley, P. F., Piersma, T., Dietz, M. W., Tang, S., Dekinga, A. and Hulsman, K. (2000). Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proc. R. Soc. Lond. B* **267**, 191-195.

Bauchinger, U., Wohlmann, A. and Biebach, H. (2005). Flexible remodeling of organ size during spring migration of the garden warbler (*Sylvia borin*). *Zool.* **108**, 97-106.

Bauchinger, U., Kolb, H., Afik, D., Pinshow, B. and Biebach, H. (2009). Blackcap warblers maintain digestive efficiency by increasing digesta retention time on the first day of migratory stopover. *Physiol. Biochem. Zool.* **82**, 541-548.

Biebach, H. (1998). Phenotypic organ flexibility in garden warblers *Sylvia borin* during long-distance migration. *J. Avian Biol.* **29**, 529-535.

Biebach, H., Friedrich, W. and Heine, G. (1986). Interaction of bodymass, fat, foraging and stopover period in trans-Sahara migrating passerine birds. *Oecologia* **69**, 370-379.

Biebach, H., Biebach, I., Friedrich, W., Heine, G., Partecke, J. and Schmidl, D. (2000). Strategics of passerine migration across the Mediterranean Sea and the Sahara Desert: a radar study. *Ibis* **142**, 623-634.

Blem, C. R. (1976). Patterns of lipid storage and utilization in birds. *Amer. Zool.* **16**, 671-684.

Carpenter, F. L., Hixon, M. A., Beuchat, C. A., Russell, R. W. and Paton, D. C. (1993). Biphasic mass gain in migrant hummingbirds: Body composition changes, torpor, and ecological significance. *Ecology* **74**, 1173-1182.

Caviedes-Vidal, E., McWhorter, T. J., Lavin, S. R., Chediack, J. G., Tracy, C. R. and Karasov, W. H. (2007). The digestive adaptation of flying vertebrates: high intestinal paracellular absorption compensates for smaller guts. *Proc. Natl. Acad. Sci. USA* **104**, 19132-19137.

Chang, M. H. and Karasov, W. H. (2004). How the house sparrow *Passer domesticus* absorbs glucose. *J. Exp. Biol.* **207**, 3109-3121.

Chediack, J. G., Caviedes-Vidal, E., Karasov, W. H. and Pestchanker, M. (2001). Passive absorption of hydrophilic carbohydrate probes by the house sparrow *Passer domesticus*. *J. Exp. Biol.* **204**, 723-731.

Chediack, J. G., Caviedes-Vidal, E., Fasulo, V., Yamin, L. J. and Karasov, W. H. (2003). Intestinal passive absorption of water-soluble compounds by sparrows: effect of molecular size and luminal nutrients. *J. Comp. Physiol. B* **173**, 187-197.

Cramp, S. (1992). *The Birds of Western Palearctic*. Oxford: Oxford University Press.

Crowley, P. H. (1992). Resampling methods for computation-intensive data analysis in ecology and evolution. *Ann. Rev. Ecol. System.* **23**, 405-447.

Diamond, J. (1991). Evolutionary design of intestinal nutrient absorption: enough but not too much. *News Physiol. Sci.* **6**, 92-96.

Fasano, A. (2008). Physiological, pathological and therapeutic implications of zonulin-mediated intestinal barrier modulation. *Am. J. Pathol.* **173**, 1243-1252.

Gannes, L. Z. (2002). Mass change pattern of blackcaps refueling during spring migration: evidence for physiological limitations to food assimilation. *Condor* **104**, 231-239.

Gill, F. B. (2007). *Ornithology*. New York: W. H. Freeman and Company.

Hume, I. D. and Biebach, H. (1996). Digestive tract function in the long-distance migratory garden warbler, *Sylvia borin*. *J. Comp. Physiol. B* **166**, 388-395.

Izhaki, I. and Maitav, A. (1998). Blackcaps *Sylvia atricapilla* stopping over at the desert edge; inter- and intra-sexual differences in spring and autumn migration. *Ibis* **140**, 234-243.

Karasov, W. H. and Cork, S. J. (1994). Glucose absorption by a nectarivorous bird: the passive pathway is paramount. *Am. J. Physiol.* **267**, G16-G26.

Karasov, W. H. and Pinshow, B. (1998). Changes in lean mass and in organs of nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. *Physiol. Zool.* **71**, 435-448.

Karasov, W. H. and Pinshow, B. (2000). Test for physiological limitation to nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. *Physiol. Biochem. Zool.* **73**, 335-343.

Karasov, W. H., Pinshow, B., Starck, J. M. and Afik, D. (2004). Anatomical and histological changes in the alimentary tract of migrating blackcaps (*Sylvia atricapilla*): a comparison among fed, fasted, food-restricted and refed birds. *Physiol. Biochem. Zool.* **77**, 149-160.

Klaassen, M. and Biebach, H. (1994). Energetics of fattening and starvation in the long-distance migratory garden warbler, *Sylvia borin*, during the migratory phase. *J. Comp. Physiol. B* **164**, 362-371.

Klaassen, M., Kvist, A. and Lindström, A. (2000). Flight costs and fuel composition of a bird migrating in a wind tunnel. *Condor* **102**, 444-451.

Lavee, D. and Safriel, U. N. (1989). The dilemma of cross-desert migrants-stopover or skip a small oasis? *J. Arid Environ.* **17**, 69-81.

Lavee, D., Safriel, U. N. and Meilijson, I. (1991). For how long do trans-Saharan migrants stop over at an oasis? *Ornis Scandinavica* **22**, 33-44.

Levey, D. J. and Cipollini, M. L. (1996). Is most glucose absorbed passively in northern bobwhite? *Comp. Biochem. Physiol.* **113A**, 225-231.

López-Calleja, M. V., Bozinovic, F. and Martínez del Rio, C. (1997). Effects of sugar concentration on hummingbird feeding and energy use. *Comp. Biochem. Physiol.* **118A**, 1291-1299.

McWhorter, T. J. (2005). Paracellular intestinal absorption of carbohydrates in mammals and birds. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (eds J. M. Starck and T. Wang), pp. 113-140. Enfield, New Hampshire: Science Publishers.

McWhorter, T. J. and Karasov, W. H. (2007). Paracellular nutrient absorption in a gum-feeding New World primate, the common marmoset *Callithrix jacchus*. *Am. J. Primatol.* **69**, 1399-1411.

McWhorter, T. J., Hartman Bakken, B., Karasov, W. H. and Martínez del Rio, C. (2006). Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. *Biol. Lett.* **2**, 131-134.

Napier, K. R., Purchase, C., McWhorter, T. J., Nicolson, S. W. and Fleming, P. A. (2008). The sweet life: diet sugar concentration influences paracellular glucose absorption. *Biol. Lett.* **4**, 530-533.

Pappenheimer, J. R. (1993). On the coupling of membrane digestion with intestinal absorption of sugars and amino acids. *Am. J. Physiol.* **265**, G409-G417.

Payevsky, V. A. (2000). Rybachy-type trap. In *Bird Station Manual* (ed. P. Busse), pp. 20-24. Gdańsk: Gdańsk University Press.

Pennycuick, C. J. and Battley, P. F. (2003). Burning the engine: a time-marching computation of fat and protein consumption in a 5420-km non-stop flight by great knots, *Calidris tenuirostris*. *Oikos* **103**, 323-332.

Piersma, T. (1998). Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? *J. Avian Biol.* **29**, 511-520.

Piersma, T. and Gill, R. E. J. (1998). Guts don't fly: small digestive organs in obese bartailed godwits. *Auk* **115**, 196-203.

Quinn, G. P. and Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.

Robertson, C. (1991). Computationally intensive statistics. In *New Developments in Statistics for Psychology and the Social Sciences*, vol. 2 (eds P. Lovie and A. D. Lovie), pp. 49-80. London: bpS and Routledge.

Shirihai, H. (1996). *The Birds of Israel*. London: Academic Press.

Shirihai, H., Gargallo, G. and Helbig, A. J. (2001). *Sylvia Warblers. Identification, Taxonomy and Phylogeny of the Genus Sylvia*. London: A and C Black.

Sibly, R. M. (1981). Strategies of digestion and defecation. In *Physiological Ecology: An Evolutionary Approach to Resource Use* (eds C. R. Townsend and P. Calow), pp. 109-139. Oxford: Blackwell Scientific Publications.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*. New York: W. H. Freeman.

Tracy, C. R., McWhorter, T. J., Korine, C., Wojciechowski, M. S., Pinshow, B. and Karasov, W. H. (2007). Absorption of sugars in the Egyptian fruit bat (*Rousettus aegyptiacus*): a paradox explained. *J. Exp. Biol.* **210**, 1726-1734.

Welling, P. G. (1986). *Pharmacokinetics: Processes and Mathematics*. Washington, D.C.: American Chemical Society.

Wojciechowski, M. S., Yosef, R. and Pinshow, B. (2005). Body composition of small migrating birds at a stopover site in southern Israel. *Integr. Comp. Biol.* **44**, 666.