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RESEARCH ARTICLE

The cost of digestion in the fish-eating myotis (*Myotis vivesi*)

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ABSTRACT

Flying vertebrates, such as bats, face special challenges with regards to the throughput and digestion of food. On the one hand, as potentially energy-limited organisms, bats must ingest and assimilate energy efficiently in order to satisfy high resting and active metabolic demands. On the other hand, the assimilation of nutrients must be accomplished using a digestive tract that is, compared with that of similarly sized non-flying vertebrates, significantly shorter. Despite these competing demands, and the relative breadth of dietary diversity among bats, little work has been done describing the cost of digestion, termed 'specific dynamic action' (SDA). Here, we provide the first systematic assessment of the SDA response in a bat, the fish-eating myotis (*Myotis vivesi*). Given the shorter digestive tract and the relatively higher resting and active metabolic rates of bats in general, and based on anecdotal published evidence, we hypothesized that the SDA response in fish-eating myotis would be dependent on meal size and both significantly more brief and intense than in small, non-flying mammals. In agreement with our hypothesis, we found that the peak metabolic rate during digestion, relative to rest, was significantly higher in these bats compared with any other mammals or vertebrates, except for some infrequently eating reptiles and amphibians. Additionally, we found that the magnitude and duration of the SDA response were related to meal size. However, we found that the duration of the SDA response, while generally similar to reported gut transit times in other small bats, was not substantially shorter than in similarly sized non-flying mammals.

KEY WORDS: Specific dynamic action, Bat, Oxygen consumption, Metabolic cost

INTRODUCTION

The evolution of flight in vertebrates was accompanied by changes not just in the musculoskeletal, circulatory and respiratory systems but also in the digestive system. Generally, volant vertebrates have much shorter digestive tracts than their similarly sized non-volant terrestrial counterparts (Caviedes-Vidal et al., 2007). It has been postulated that reductions in the size of the gut were selectively advantageous because of the weight savings they afforded flying vertebrates (Caviedes-Vidal et al., 2007). However, flight is the most energetically expensive form of locomotion and birds and bats must achieve high rates of energy assimilation to fuel locomotion. For this reason, the digestive function of flying vertebrates has long been of interest to comparative physiologists. Digestive performance is also a potential determinant of foraging behaviour. Particularly in energy-limited birds and bats, the rate of digestion may constrain food intake rate. For example, nectarivorous birds (Martinez del Rio et al., 2001) and bats (Martinez del Rio et al., 2001; Ramírez P. et al., 2005) fed dilute sugar solutions may lose mass, presumably because digestion-limited maximal feeding rates cannot compensate for insufficient energy density. However, digestive costs for nectarivorous birds and bats would probably be relatively small (but see Mata, 2010), as simple carbohydrate meals are comparatively easy to digest (McCue, 2006) and much of hexose absorption across the gut wall occurs passively in these animals (Caviedes-Vidal et al., 2007; McWhorter et al., 2006; Tracy et al., 2007).

Some aspects of foraging energetics, behaviour and ecology are relatively well studied in bats. For example, a good deal of work has been done on characterizing the cost of flight and thermogenesis in bats (Kurta et al., 1987; Morris et al., 1994; Norberg et al., 1993; Welch et al., 2008; Winter and von Helversen, 1998; Winter et al., 1998). However, despite its importance to energetics and foraging behaviour, remarkably little attention has been given to the energetic cost of ingesting, digesting and assimilating a meal, termed 'specific dynamic action' (SDA). Only a few studies have explicitly examined metabolic rate in bats after they have been fed and while they are otherwise relatively inactive (Matheson et al., 2010; Morris et al., 1994; Riedesel and Williams, 1976). In each of these cases, determination of at least some features of the SDA response is impossible because appropriate controls (e.g. sham feeding) were not conducted, pre-feeding metabolic rates were not recorded, or bats entered torpor at some point during the study period, prohibiting determination of post-prandial normothermic metabolic rate. However, in at least two of these studies, the authors reported that the metabolic rate of bats was elevated multifold over presumed resting values during the initial stages of the SDA response (Morris et al., 1994; Riedesel and Williams, 1976). The magnitude of this apparent response is striking. Generally, metabolic rates during the SDA response peak at values $\leq$2 times that of resting metabolic rate (RMR) in mammals (McCue, 2006; Secor, 2009). However, published reviews of the SDA response do not include any data from bats.

The lack of research on digestive costs in bats is unfortunate, because there is a great deal of dietary diversity in this vertebrate order, including nectarivores, frugivores, insectivores, carnivores and, perhaps most famously, the haematophagous diet of vampire bats. Researchers have long understood that the composition of a meal influences the cost of digestion. Specifically, ingestion of protein-rich meals elicits substantially higher SDA responses than ingestion of carbohydrate- or fat-rich meals (McCue, 2006; Secor, 2009). Thus, the broad variation in diet among bats suggests that the relative cost of digestion may vary substantially as well.

The fish-eating myotis (*Myotis vivesi*; Ménégaux 1901) specializes on a highly protein-rich diet of small marine crustaceans, fish and some insects (Otálora-Ardila et al., 2013). Their unusual diet has

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Received 28 October 2014; Accepted 18 February 2015

The Journal of Experimental Biology

The Journal of Experimental Biology (2015) 218, 1180-1187 doi:10.1242/jeb.115964

List of symbols and abbreviations

MR_{kJ}	metabolic rate as the oxy-joule equivalent of $\dot{V}_{O_2}$ (kJ h^{-1})
Q_{2x}	response coefficient
RER	respiratory exchange ratio: the ratio of carbon dioxide production rate to oxygen consumption rate
RMR	resting metabolic rate (ml O_2 h^{-1})
SDA	specific dynamic action: the metabolic energy expended on meal ingestion, digestion and assimilation
$\dot{V}_{CO_2}$	carbon dioxide production rate (ml h^{-1})
$\dot{V}_{O_2}$	oxygen consumption rate (ml h^{-1})
$\dot{V}_{O_2}$,norm	oxygen consumption rate normalized to pre-feeding RMR

helped earn these bats placement on the International Union for Conservation of Nature (IUCN) red list of threatened species (Arroyo-Cabrales and Álvarez Castañeda, 2014; Hutson et al., 2001). These bats potentially face competing constraints in digesting and assimilating meals. Like other bats (Caviedes-Vidal et al., 2007), fish-eating myotis probably possess digestive tracts that are shorter than those found in similarly sized, non-flying mammals. Yet, a protein-rich diet is associated with a greater cost of digestion than a lipid- or carbohydrate-rich diet (Secor, 2009). Thus, these bats are faced with a potentially large digestive challenge and reduced digestive machinery, and possibly a compressed gut transit duration with which to meet this challenge.

We sought to characterize the metabolic rate of fish-eating myotis bats both at rest and during the digestion of a meal. We fed bats meals of shrimp meat of two different sizes (1.5 and 3.0 g) and measured oxygen consumption and carbon dioxide production in order to investigate the magnitude of the SDA response in relation to meal mass. Given their protein-rich diet and comparatively short digestive tracts (Caviedes-Vidal et al., 2007), and because the limited available evidence suggests peak postprandial metabolic rates in bats are relatively large (Morris et al., 1994; Riedesel and Williams, 1976), we hypothesized that the SDA response in fish-eating myotis bats would be brief and intense. Specifically, we predicted that the peak metabolic rates exhibited during the SDA response would be both a function of meal size and proportionately greater than in non-volant mammals (≫200%). Additionally, we predicted that the duration of the SDA response would be related to meal size, but in both cases less than 3 h, the average minimum SDA duration reported for comparably sized non-flying mammals (McCue, 2006; Secor, 2009).

RESULTS

RMR

Averaged across all bats and all trials, pre-feeding RMR was 25.9±2.9 ml O_2 h^{-1}. RMR variation among meal size treatments offered was nearly significant (sham-feeding: 29.7±7.9 ml O_2 h^{-1}; 1.5 g: 25.3±2.1 ml O_2 h^{-1}; 3.0 g: 22.8±4.9 ml O_2 h^{-1}; $F_{1,11}$=4.5454, P=0.0564; Table 1, Fig. 1). RMR values decreased slightly, but significantly, over the hour prior to feeding ($F_{1,35}$=20.2201, P<0.0001). Because bats were both acclimatized to chambers on several days prior to trials and placed in the chambers a minimum of 1 h prior to recording on a given trial night, it is surprising that this temporal variation in RMR was observed. We hypothesize that the declining oxygen consumption rate ($\dot{V}_{O_2}$) over time during this period may have been due to modest stress associated with the noise of switching between reference and experimental respirometric chambers at the beginning of each hour of recording. Proceeding with this assumption, we used RMR values for each bat averaged over the hour prior to feeding (mean of three measurements at binned time points: −50, −30, −10 min relative to feeding) for further calculations as it was reasonable to expect a similar, minor temporal effect of stress on $\dot{V}_{O_2}$ at the beginning of each subsequent dwell period.

SDA response

In every 1.5 or 3.0 g feeding trial, and most sham-feeding trials, $\dot{V}_{O_2}$ rose sharply above RMR levels immediately following food administration. In most instances, $\dot{V}_{O_2}$ readings peaked during the first or second 20 min time bin following feeding (sham-feeding: 27±41 min; 1.5 g: 13±8 min; 3.0 g: 23±24 min; Table 1, Fig. 1A). The time, in minutes, from feeding to the peak $\dot{V}_{O_2}$ (time to peak) did not differ significantly among trial types ($F_{1,11}$=0.04453, P=0.8367; Table 1). Average peak post-feeding or sham-feeding $\dot{V}_{O_2}$ values were significantly greater than RMR and varied with trial type (sham-feeding: 49.1±18.7 ml O_2 h^{-1}; 1.5 g: 83.2±7.0 ml O_2 h^{-1}; 3.0 g: 103.0±14.8 ml O_2 h^{-1}; $F_{1,11}$=43.092; P<0.0001; Table 1, Fig. 1A).

The rise in $\dot{V}_{O_2}$ following the sham feedings confirmed that the bats exhibited a stress response as a result of interaction with the researcher during feeding. We corrected for this effect of stress in order to more accurately quantify the SDA response by subtracting elevated post-feeding $\dot{V}_{O_2}$ and carbon dioxide production rate ($\dot{V}_{CO_2}$) during the sham-feeding trial from corresponding data recorded during the 1.5 and 3.0 g trials (see Materials and methods). The corrected $\dot{V}_{O_2}$ values peaked slightly later than raw values (1.5 g: 37±24 min; 3.0 g: 30±25 min; Table 1, Fig. 1B), though there was still no significant difference in timing between 1.5 and 3.0 g feedings (paired t_5=0.7906, P=0.4650). As with raw values, the absolute magnitude of peak corrected $\dot{V}_{O_2}$ was significantly greater following the 3.0 g feeding compared with the 1.5 g feeding (1.5 g: 79.0±7.1 min; 3.0 g: 102.2±14.7 min; paired t_5=−7.0844, P=0.0009; Table 1, Fig. 1B). The scope of the peak SDA response (the peak metabolic rate expressed as a factor of the pre-feeding RMR) was similarly significantly different, with postprandial $\dot{V}_{O_2}$ increasing to 3.0±0.1 times and 4.3±0.3 times pre-feeding RMR following 1.5 and 3.0 g feedings, respectively (paired t_5=−3.2744, P=0.0221; Table 1, Fig. 2). Additionally, the duration of the SDA response, defined as the period where post-feeding $\dot{V}_{O_2}$ remained more than 1 s.d. above the average pre-feeding RMR, was significantly greater when bats were fed 3.0 g of shrimp meat compared with 1.5 g (1.5 g: 193±12 min; 3.0 g: 273±10 min; paired t_5=−4.6710, P=0.0055; Table 1, Fig. 1B).

We calculated the total oxy-joule equivalent of the SDA as the integrated area under the curve after subtracting the running RMR value. The SDA was approximately twice as great when bats ingested a 3.0 g meal (3.50±0.11 kJ) compared with a 1.5 g meal (1.72±0.08 kJ; paired t_5=−13.2198, P<0.0001; Table 1). Correspondingly, the SDA coefficient (the ratio of SDA to meal energy, the energy content of the ingested meal) was nearly identical between the two trials (1.5 g: 19.8±0.9%; 3.0 g: 20.1±0.7%; paired t_5=−0.3259, P=0.7577; Table 1).

RER

The respiratory exchange ratio (RER), the ratio of $\dot{V}_{CO_2}/\dot{V}_{O_2}$, was calculated for each binned time period. Prior to feeding, RER values in bats averaged 0.77±0.01, 0.78±0.00 and 0.77±0.01 during sham-feeding, 1.5 g and 3.0 g trials, respectively (Table 1, Fig. 3). RER did not vary significantly among trials ($F_{1,11}$=0.0561, P=0.8172) or as a function of time bin during the pre-feeding period ($F_{1,11}$=0.0158, P=0.9023). Immediately following feeding, RER

The Journal of Experimental Biology (2015) 218, 1180-1187 doi:10.1242/jeb.115964

Table 1. Summary of treatment parameters and resulting features of the digestive response in fish-eating myotis bats (*Myotis vivesi*; *N*=6)

	Treatment					
Variables	Sham feeding	1.5 g meal	3.0 g meal	Q_{2x}	*F* or *t*	*P*
N	6	6	6			
Meal mass (g)	0	1.5	3.0			
Meal size (% body mass)	0	5.3±0.1%	10.4±0.3%			
Meal energy (kJ)	0	8.69	17.39			
Raw data						
RMR (ml O_2 h^{-1})	29.7±7.9	25.3±2.1	22.8±4.9		$F_{1,11}$=4.5454	0.0564
RMR at –50, –30, –10 min before feeding					$F_{1,35}$=20.2201	**<0.0001**
Peak MR (ml O_2 h^{-1})	49.1±18.7	83.2±7.0	103.0±14.8		$F_{1,11}$=43.092	**<0.0001**
Time to peak (min)	27±41	13±8	23±24		$F_{1,11}$=0.04453	0.8367
Corrected data						
Peak MR (ml O_2 h^{-1})	–	79.0±7.1	102.2±14.7	1.3±0.0	t_5=–7.0844	**0.0009**
Scope	–	3.0±0.1	4.3±0.3	1.4±0.1	t_5=–3.2744	**0.0221**
Time to peak (min)	–	37±24	30±25		t_5=0.7906	0.465
Duration (min)	–	193±12	273±10	1.4±0.1	t_5=–4.671	**0.0055**
SDA (kJ)	–	1.72±0.08	3.50±0.11	2.05±0.11	t_5=–13.2198	**<0.0001**
SDA coefficient	–	19.8±0.9%	20.1±0.7%		t_5=–0.3259	0.7577

Raw data refer to absolute values obtained during each treatment type. Corrected values refer to respirometric data collected during the 1.5 g and 3.0 g treatments that have been adjusted to account for the elevated metabolic response observed in the control, sham-fed group. Summary statistics for dependent variables are presented. All data are presented as means±s.e.m.

RMR, relative metabolic rate; MR, metabolic rate; SDA, specific dynamic action; Q_{2x}, response coefficient.

values decreased in bats during each trial, averaging 0.70±0.01, 0.66±0.01 and 0.69±0.01 during the initial time bin for sham-feeding, 1.5 g and 3.0 g trials, respectively (Table 1, Fig. 3). Subsequently, RER values generally rose, most quickly in the sham-fed bats, and most slowly in the bats fed 3.0 g of food. RER values generally returned to pre-feeding levels at roughly the same time $\dot{V}_{O_2}$ values returned to pre-feeding levels.

DISCUSSION

The RMR observed in fasted, fish-eating myotis bats was approximately 25–30% lower than reported basal metabolic rates in similarly sized phylostomid bats (Cruz-Neto et al., 2001). This is somewhat surprising given an endotherm's RMR is expected to be higher than its basal metabolic rate because of additional thermoregulatory costs. However, the measurements reported here

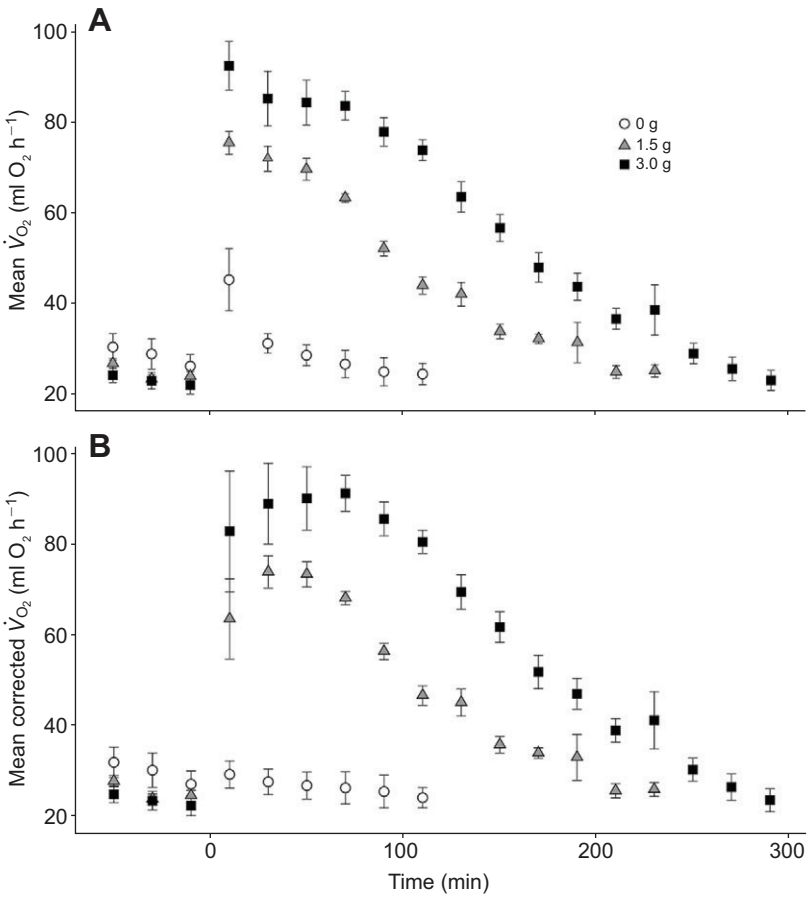

Fig. 1. Preprandial and postprandial oxygen consumption in resting fish-eating myotis (*Myotis vivesi*) as a function of the size of meal eaten. The meal, composed of 1.5 or 3.0 g of white shrimp meat, was administered at time 0. In sham-feeding trials (0 g food), respirometry chambers were opened and forceps were introduced in a manner similar to that during feeding trials except that no food was given. (A) Whole-animal oxygen consumption rates ($\dot{V}_{O_2}$) for each time bin. (B) Whole-animal $\dot{V}_{O_2}$ for each time bin corrected by subtracting the excess oxygen consumption observed in sham-fed bats that was assumed to be related to handling stress and not food processing, digestion and absorption. The remaining excess postprandial oxygen consumption comprises the specific dynamic action (SDA) response. Values are means±s.e.m. for six individuals.

The Journal of Experimental Biology (2015) 218, 1180-1187 doi:10.1242/jeb.115964

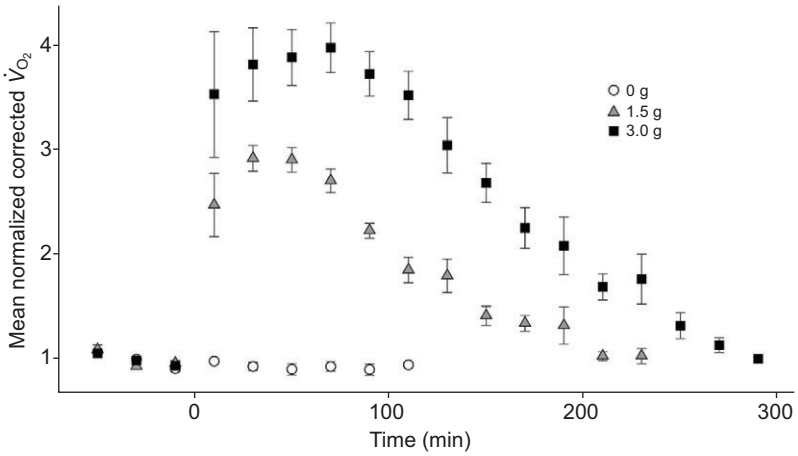

Fig. 2. Preprandial and postprandial oxygen consumption, normalized to preprandial relative metabolic rate (RMR), in resting fish-eating myotis (*M. vivesi*) as a function of the size of meal eaten. Details of the meal and sham-feeding trials are the same as in Fig. 1. Values are means±s.e.m. for 6 individuals.

were obtained while bats were held at a relatively warm temperature of ∼27°C. While the lower critical temperature (the temperature that defines the lower bound of an animal's thermoneutral zone) of the fish-eating myotis is not known, it is probably close to 27°C. Morris et al. (1994) reported that the lower critical temperature of the smaller (8–10.5 g) Gould's long-eared bat is 30°C. It is puzzling that average pre-feeding RMR values differed among treatment groups to a magnitude that was nearly significant. Bats were subjected to each treatment in a random order. Thus, we do not suspect variation is systematically related to a change in RMR within individuals over time resulting from de-training in captivity or increasing acclimatization to the respirometry chamber.

In contrast to our predictions, the duration of the SDA response in these bats was not significantly shorter than duration values reported among mammals, though it was near the shorter durations reported in similarly sized mammals (McCue, 2007; Secor, 2009). Duration values reported here agree well with apparent gut transit times (i.e. time to first appearance of faeces from a meal) in other small bats fed comparable arthropod meals. Relatively rapid gut transit times would be predicted in small bats as a result of their comparatively short gut tract lengths and high metabolic rates (Caviedes-Vidal et al., 2007). In several studies on bats smaller than the fish-eating myotis, transit times varied from ∼45 to 165 min (Buchler, 1975; Grant, 1988; Luckens et al., 1971). Transit time was related to activity level while digesting, with transit times in 'quiet' (resting) bats being 2–4 times as long as those in actively moving little brown bats (Buchler, 1975). Our bats were constrained in respirometry chambers and considered otherwise at rest. Further, gut transit times appear to scale positively with body mass in bats (Buchler, 1975). Thus, taken as a whole, average duration values of 193±12 and 273±10 min in fish-eating myotis fed 1.5 and 3.0 g of food appear to agree quite well with probable gut transit times. Unfortunately, because the timing of appearance of faeces could not be determined without disturbing bats, actual gut transit times in our fish-eating myotis remain unquantified.

As predicted, and as has been shown repeatedly in other vertebrates (McCue, 2006; Secor, 2009), meal size influenced both the magnitude and duration of the SDA response in fish-eating myotis bats. As we hypothesized, the scope of the SDA response observed was significantly higher than that observed in non-volant terrestrial mammals (McCue, 2006; Secor, 2009). Scope values in non-volant mammals are typically less than 2. Because both meal size and composition have known effects on SDA, it is possible that variation in one or both of these meal parameters among this and other mammalian SDA response studies accounts for the difference. In our study, bats were given relatively large (5% or 10% of body mass), protein rich (>90% protein by dry mass; see Materials and methods) meals. Many studies on mammals and birds employ meal sizes that constitute substantially less than 5% of the animal's body mass and it is reasonable to expect lower scope values in these cases (McCue, 2006; Secor, 2009). However, even in the several studies where mammals were fed meals constituting >5% of body mass,

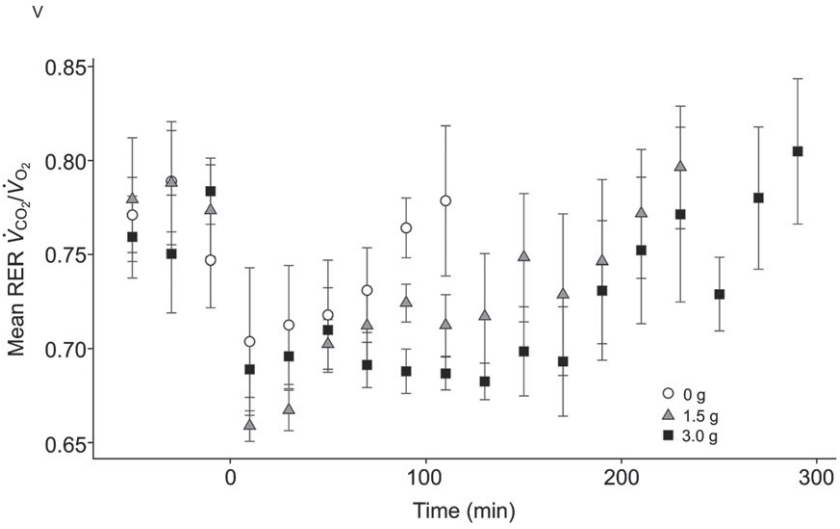

Fig. 3. Preprandial and postprandial respiratory exchange ratio (RER) in resting fish-eating myotis (*M. vivesi*) as a function of the size of meal eaten. Details of the meal and sham-feeding trials are the same as in Fig. 1. Values are means±s.e.m. for six individuals.

The Journal of Experimental Biology (2015) 218, 1180-1187 doi:10.1242/jeb.115964

scope values still ranged between 1.36 and 2.02, considerably lower than the scopes observed in this study (Atkinson and Lusk, 1919; Benedict and Pratt, 1913; Campbell et al., 2000; Costa and Kooyman, 1984; Curcio et al., 1999; Forbes et al., 1934; Hindle et al., 2003; Kriss, 1938; Weiss and Rapport, 1924; Williams et al., 1912). Because the peak of the SDA response is typically greater when digesting protein-rich meals compared with equicaloric lipid- or carbohydrate-rich meals (McCue, 2006; Secor, 2009), it is possible the highly protein-rich shrimp diet offered to our bats is, in part, responsible for the greater scope values seen, relative to other mammalian studies. Indeed, the coefficient values of ∼20% are double the average value reported for mammals as a group (Secor, 2009). However, most of the studies noted above that offered comparably sized meals offered protein-rich foods as well (e.g. earthworms or 'meat') and coefficient values for most of these were well below 20%. Thus, it seems that diet composition is not sufficient to explain the greater scope values observed. Notably, the scope values we observed in fish-eating myotis bats are substantially higher than those observed in birds (also generally <2; McCue, 2006; Secor, 2009). Scope values in the fish-eating myotis, particularly following the 3.0 g meal, were similar to those seen in many reptiles or amphibians, including pythonid snakes (McCue, 2006; Secor, 2009). When fed meals equaling 5% and 10% of their body mass, fish-eating myotis bats exhibited scope values quite similar to those exhibited by marine toads (*Bufo marinus*) and Ambystomatid salamanders fed comparably sized meals (Secor and Boehm, 2006; Secor and Faulkner, 2002). However, scope values observed in fish-eating myotis were somewhat lower than values observed in the Burmese python (*Python molurus*) fed meals of 5% or 15% of their body mass (Secor and Diamond, 1997). Further, they were similar to apparent values recorded in the cave myotis (*Myotis velifer*) (Riedesel and Williams, 1976) and Gould's long-eared bat (*Nyctophilus gouldi*) (Morris et al., 1994), though exact scope values were not determined in these studies. Compared with these bats, however, the duration of the SDA response in the fish-eating myotis appeared shorter, although duration estimates were not given for *M. velifer*.

It is unclear why the peak magnitude of the SDA response observed in fish-eating myotis bats should be so great. If the evolution of shorter digestive tracts in flying vertebrates were the explanation because this meant more rapid gut transit times, then we might expect the increased intensity of the SDA response was necessary to achieve digestion given the shorter available window. Yet, duration values reported here are not shorter than those observed in small non-flying mammals (e.g. 4 h in the 40–70 g star-nosed mole; Campbell et al., 2000). Further, the scope of the SDA response in birds is much lower than that in fish-eating myotis bats (Secor, 2009) despite similarly reduced digestive tract lengths (Caviedes-Vidal et al., 2007). Explanations for the large scope of the SDA response in pythonid snakes and some other reptiles or amphibians can be attributed to their normally low RMRs and the infrequency with which they feed. In pythonid snakes in particular, digestion of substantial meals involves the hypertrophy of multiple digestive and support tissues, further elevating digestive costs (McCue, 2006; Secor, 2003, 2009). In contrast, bats typically have relatively elevated RMRs, even compared with those of similarly sized non-volant mammals. Further, bats must feed regularly and frequently to sustain themselves. Thus, the explanations that apply to some reptiles and amphibians cannot justify the large peak in metabolic rates observed during the SDA in bats.

The overall SDA, like the scope and duration of the response, was also dependent on the meal size, as expected. The proportional increase in peak $\dot{V}_{O_2}$, scope and duration of the SDA response associated with the doubling of meal size (the response coefficient, Q_{2x}; *sensu* Secor, 2009) averaged 1.3–1.4 in fish-eating myotis (1.3±0.0, 1.4±0.1 and 1.4±0.1, respectively; Table 1). As the SDA is a product of both the duration and magnitude of the SDA response, the Q_{2x} of the SDA was, not surprisingly, larger (2.05±0.11; Table 1). The approximate doubling of SDA with a doubling of meal size agrees well with average changes in SDA seen in most other animal groups (Secor, 2009). Because the energy content of the 3.0 g meal was exactly twice that of the 1.5 g meal, this resulted in a SDA coefficient that was independent of meal size. At approximately 20%, the SDA coefficient observed in fish-eating myotis was similar to that observed in many reptiles and significantly higher than that in many birds and mammals, particularly those fed more easily digested carbohydrate- or fat-rich meals (McCue, 2006; Secor, 2009). Using bomb calorimetry to determine the energy content of prey items and residual energy content of faeces, researchers have determined apparent digestive efficiency in several species of insectivorous myotis bats (Barclay et al., 1991; O'Farrell et al., 1971). Digestive efficiency varied with prey type (e.g. moth versus mealworm larvae). Yet, when comparing similar prey items, digestive efficiency was quite comparable across species (Barclay et al., 1991). In their study, Barclay et al. found digestive efficiency was higher when bats were fed mealworm larvae (88–90%) compared with when they were fed moths (75–78%). Because we did not collect faeces, we cannot determine the energy content remaining following digestion of shrimp by our bats. Using the values obtained by Barclay et al. (1991), we can calculate that fish-eating myotis achieved a net energy intake (i.e. meal energy – SDA) of 5.23–6.27 and 10.42–12.51 kJ when offered a meal of 1.5 or 3.0 g of shrimp meat, respectively. Given the bats were fed shrimp meat from which legs, head and the shell were already removed, it is safe to assume this meal was comparatively digestible. Thus, it is likely the net energy intake was near the upper end of these ranges.

In contrast to patterns seen in most studies of the digestive response of vertebrates, RER values in fed fish-eating myotis bats initially declined following feeding. Typically, the release of protons by parietal cells to acidify the contents of the lumen of the stomach is accompanied by a release of bicarbonate into the circulation, subsequently raising the pH of the blood (Niv and Fraser, 2002). This 'alkaline tide', detectable in most post-prandial animals, can promote an increase in the flux of CO_2 from the body across the respiratory exchange surfaces above that which reflects the increase in CO_2 production associated with increased O_2 consumption. In many animals, including bats (Morris et al., 1994), this increased $\dot{V}_{CO_2}$ outpaces the rise in $\dot{V}_{O_2}$ that comprises the SDA response, resulting in an increase in the RER of post-prandial vertebrates compared with pre-feeding values. Yet, in fish-eating myotis bats, the increase in $\dot{V}_{O_2}$ following feeding outpaced the increase in $\dot{V}_{CO_2}$ and RER declined. We cannot, at present, explain this decrease in RER and its subsequent return to pre-feeding values following the SDA response.

Summary

Overall, this study indicates that fish-eating myotis pay a high cost in digesting a crustacean meal. Importantly, the duration of the SDA response is not significantly shorter than that seen in other, non-flying, small mammals, as might have been predicted based on their relatively short digestive tracts. The duration of the SDA response and its relatively high cost suggest that the rate of prey digestion may limit food intake rate while the high cost and high coefficient values

The Journal of Experimental Biology

The Journal of Experimental Biology (2015) 218, 1180-1187 doi:10.1242/jeb.115964

constrain net energy assimilation. As such, the digestive physiology of these bats may play a more prominent role in defining net energy intake and foraging behaviour compared with most other mammals or birds.

The diet of fish-eating myotis varies seasonally, with near-complete reliance on fish occurring in the autumn (September) and near-complete reliance on crustaceans in the winter (December; Otálora-Ardila et al., 2013). Additional studies are needed to confirm that prey type influences the magnitude of SDA and coefficient values in fish-eating myotis. However, compared with protein-rich meals, more lipid-rich prey, such as fish, generally incur more modest SDA responses in most vertebrates (Secor, 2009). Thus, the relative role SDA may play in the energy budget of fish-eating myotis may vary seasonally, making consideration of the cost of digestion even more important in understanding their ecology. For example, strong winds, strong surf and low ambient temperature during winter months may limit fish-eating myotis foraging behaviour at the time of year when they rely most on a crustacean diet. Fish-eating myotis employ torpor during this period of potentially restricted food intake (Salinas R. et al., 2014). Both heterothermy and low energy reserves may impact digestive effort at the time when relative digestive costs may be greatest.

More generally, the broad dietary variation, relatively small size and energetically expensive locomotor behaviour of bats should make them a powerful system within which to improve understanding of how digestive physiology and behaviour and ecology are linked and co-evolve. The findings described here confirm the importance of the cost of digestion as a factor crucial to understanding overall energy budgets in bats.

MATERIALS AND METHODS

Animals

Six adult fish-eating myotis (*Myotis vivesi*; Vespertlionidae; four males, two females) were collected from roost sites on Partida Norte Island in the Gulf of California (Otálora-Ardila et al., 2013). Individuals were captured in October 2013 and transported to a study facility at Colima, Mexico. Data were collected in October and November 2013. Bats were communally housed in a large nylon enclosure outside, under shade. Individuals were identified through a unique pattern of dots on their torso made by shaving small (0.5×0.5 cm) areas of fur. Each evening, bats were individually removed from the enclosure, examined, weighed and either returned to the enclosure, where food (commercially available white shrimp, provided *ad libitum* with water) was made available, or placed in metabolic chambers (see below).

The mass of each bat was recorded immediately prior to the beginning of data collection each night. Mean (±s.e.m.) mass of the bats used was 28.83±0.71 g. Temperature averaged 27.62±0.21°C across all trials, with temperatures varying by 1.07±0.15°C during trials.

Feeding trials

We evaluated the cost of digestion in fish-eating myotis bats by measuring gas exchange in fasted, resting individuals for 1 h prior to and for several hours immediately following feeding on either 1.5 or 3.0 g of commercially available white shrimp meat or a sham-feeding treatment. Shrimp meat is composed of approximately 93% protein, 2% lipid and negligible carbohydrate, by dry mass (15,270, crustaceans, shrimp, untreated, raw; USDA National Nutrient Database for Standard Reference, Release 27, 2014). The 1.5 and 3.0 g portions of shrimp meat constituted meals that were 5.3±0.1% and 10.4±0.3% of pre-feeding body mass containing 8.69 and 17.39 kJ of energy, respectively (Table 1; Krishnamoorthy et al., 1979). Each individual was subjected to each of the three treatments (1.5 or 3.0 g of food, or sham feeding) in a randomized order.

Bats were placed in respirometry chambers for 2–4 h a minimum of 3 days prior to the initiation of data collection in order to acclimate them to the surroundings and to pump noise. On the evening of data collection, a bat was placed in the chamber at least 1 h prior to the initiation of data recording. Chambers consisted of 500 ml horizontally oriented plastic cylinders. Unlike most bats, fish-eating myotis roost in cavities and crevices between and under rocks, typically adopting a horizontal orientation. Thus, the orientation of chambers used during this study permitted a natural roosting posture. Both the inlet and outlet ports entered through the chamber lid and a length of Pharmed tubing was attached to the outlet port, promoting gas mixing. Bats were loosely wrapped in a paper towel while inside the chambers. This provided a comfortable substrate on which the bats rested and helped to prevent the bat from blocking the outlet port tubing. Flow rate through all chambers was maintained at ≥500 ml min^{-1}.

Data collection periods began with a 10 min baseline recording of air drawn through an empty chamber otherwise identical to the one that contained the bat. Next, we recorded excurrent air from the experimental chamber for 50 min, followed by another 10 min baseline recording from the empty chamber. During this second baseline period, the chamber containing the bat was opened and the bat was offered pieces of shrimp meat using forceps. The bats always ate the entire 1.5 or 3.0 g meal within 3–5 min. Sham feedings were accomplished in an identical manner, with the forceps introduced into the chamber but holding nothing for approximately 3–5 min. Occasionally, bats bit at the forceps. Subsequently, the chamber lid was closed and airflow through the chamber was re-established. This process was accomplished within the time period of the concurrent 10 min baseline window. Following this second baseline and feeding event, data were recorded from the experimental chamber for 50 min, followed by a 10 min baseline recording. This recording process was repeated an additional 2–5 times, until O_2 and CO_2 traces appeared to have returned to levels similar to those in the 50 min prior to feeding.

Oxygen consumption, carbon dioxide production and water vapour were measured in excurrent air using a Sable Systems Field Metabolic System (FMS, Sable Systems International, Las Vegas, NV, USA). Data were recorded using Expedata 1.7.2 (Sable Systems International). Flow rate, O_2 and CO_2 readings were corrected for dilution effects of water vapour using equation 8.6 from Lighton (2008). CO_2 and O_2 channel recordings were corrected for lag relative to the water vapour recording channel and each was smoothed and *Z*-transformed (Bartholomew et al., 1981; Lighton, 2008). $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated using equations 11.7 and 11.8, respectively, from Lighton (2008). Because $\dot{V}_{O_2}$ values fluctuated during recordings when bats occasionally moved within the chamber, the lowest oxygen consumption averaged over a 3 min window was found within 0–20, 21–40 and 41–50 min periods of each 50 min dwell. Average $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ rates and RER (where $RER=\dot{V}_{CO_2}/\dot{V}_{O_2}$) for each 3 min window were used in subsequent analyses.

The three $\dot{V}_{O_2}$ values obtained before feeding were averaged to calculate each bat's RMR. To correct for any acute effect of the stress of researcher presence during feeding, we subtracted the post-feeding elevated metabolic rate values (the difference between $\dot{V}_{O_2}$ and RMR) observed at each time point during the sham-feeding trials from the associated time points for that bat during 1.5 and 3.0 g feeding trials. Corrected values were used for all calculations below and for comparisons between 1.5 and 3.0 g feeding trials (except for the pre-feeding RMR values). The time since feeding, rounded to the mean of the binned period, when post-feeding $\dot{V}_{O_2}$ was highest was taken as the time to peak SDA response. The duration of each bat's SDA response was calculated as the time from feeding to the first 20 min period that $\dot{V}_{O_2}$ fell to a value within 1 s.d. of the mean pre-feeding RMR. $\dot{V}_{O_2}$ values for each bat were normalized ($\dot{V}_{O_2}$,norm) by dividing by the mean RMR for that individual for that trial. The scope of the SDA response was taken as the highest $\dot{V}_{O_2}$,norm value observed for each individual during the postprandial phase. $\dot{V}_{O_2}$ values were converted to their oxy-joule equivalents (MR_{kJ} in kJ h^{-1}) via the following equation, modified from Lighton (2008):

$$MR_{kJ} = \dot{V}_{O_2} \times [16 + 5.164(RER)]. \quad (1)$$

The total cost of digestion (the SDA) was calculated by subtracting the mean pre-feeding RMR value from each post-feeding value, fitting a spline function to data from the first measurement following feeding to the first period where $\dot{V}_{O_2}$ fell to a value within 1 s.d. of the mean pre-feeding RMR, and integrating the area under this fit. The coefficient of the SDA was

calculated by dividing the total cost of digestion by the energy content of the meal (meal energy, assuming 5.796 kJ g^{-1}; Krishnamoorthy et al., 1979) and multiplying the result by 100 (McCue, 2007; Secor and Diamond, 2000).

Bat mass had no effect on the fit of any models explaining variation in metabolic variables, as revealed by similar or worse (higher) Akaike information criteria (AIC) scores compared with models lacking mass as an effect. Thus, mass was excluded as a factor in analyses. Variation in $\dot{V}_{O_2}$ and RER during each trial type was compared within and across treatments by fitting linear mixed effects models with meal size (i.e. trial type) and time (relative to feeding) as fixed effects, bat ID as a random effect and trial order (the relative day on which each of the three trial types was conducted for each bat) as a nested effect within ID. Models were fitted in R v. 3.1.0 (R Core Team, 2013) using the 'lme4' package (Bates et al., 2013) in conjunction with the 'lmerTest' package. Specific variables identified in the previous paragraph were compared between the 1.5 and 3.0 g treatments using a paired *t*-test. $P<0.05$ was considered significant. Data are presented as means±s.e.m.

Acknowledgements
Transport to Partida Norte Island was generously provided by the Secretaría de Marina-Armada de México. The Prescott College Kino Bay Center provided invaluable logistic support during fieldwork. This study was conducted under permits from Secretaría de Gobernación (no. 013/13) and from Dirección General de Vida Silvestre (01947/13) to L.G.H.M. The authors also thank three anonymous reviewers for their constructive feedback on this manuscript.

Competing interests
The authors declare no competing or financial interests.

Author contributions
J.J.F.-M. and A.O.-A. collected the experimental animals. K.C.W. and L.G.H.M. conceived of and designed the experiment. A.O.-A. and L.G.H.M. collected data. K.C.W. analysed and interpreted the data. All authors contributed to the writing of the manuscript.

Funding
Funding for this research was provided by Dirección General de Asuntos del Personal Académico [PAPIIT no. IN202113] to L.G.H.M. and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant [no. 386466] to K.C.W. L.G.H.M. was supported by sabbatical grants from Consejo Nacional de Ciencia y Tecnología [no. 232939] and from Dirección General de Asuntos del Personal Académico [PASPA 062/2014].

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