Testing the "Fasting While Foraging" Hypothesis: Effects of Recent Feeding on Plasma Metabolite Concentrations in Little Brown Bats (*Myotis lucifugus*)

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ABSTRACT

Plasma metabolite concentrations can be used to understand nutritional status and foraging behavior across ecological contexts including prehibernation fattening, migration refueling, and variation in foraging habitat quality. Generally, high plasma concentrations of the ketone β-hydroxybutyrate, a product of fat catabolism, indicate fasting, while triglycerides indicate recent feeding and fat accumulation. In recent studies of insectivorous bats, triglyceride concentration increased after feeding as expected, but β-hydroxybutyrate also unexpectedly increased rather than decreased. An aerial-hawking foraging strategy is energetically demanding, and thus it has been hypothesized that foraging by insectivorous bats requires catabolism of stored fat. We tested this hypothesis by quantifying plasma β-hydroxybutyrate and triglyceride concentration following feeding in little brown bats (*Myotis lucifugus*) that were temporarily housed in individual cages to prevent flight. We provided a fixed amount of food and collected blood samples at different intervals after feeding to produce variation in plasma metabolite concentrations. Plasma triglyceride concentration responded as predicted, but similar to previous studies and contrary to our prediction, when flight was eliminated plasma β-hydroxybutyrate concentration responded similarly to triglyceride. Thus, it is unlikely that the unexpected plasma β-hydroxybutyrate patterns observed in previous studies were related to flight. The mechanism underlying this unexpected pattern remains unknown, but the response has been consistent in all studies to date. Thus, plasma metabolite analysis provides an effective tool for studies of nutritional status, although more work is needed to understand why insectivorous bats respond differently than other taxa.

Keywords: β-hydroxybutyrate, triglyceride, aerial hawking, Chiroptera, insectivore, hibernation energetics, white-nose syndrome, feeding rate.

Introduction

Balancing the acquisition and expenditure of energy is critical for all organisms (Kronfeld-Schor and Dayan 2013), and trade-offs associated with energy balance can influence survival during periods of low resource abundance (Bailey et al. 2012; Moeller et al. 2013; Saino et al. 2014; Womble et al. 2014; Boyles et al. 2016). Techniques that can be used to estimate energy acquisition and expenditure in wild populations can aid fundamental ecological studies as well as conservation and management (Williams et al. 2007; Bailey et al. 2012). While techniques to quantify energy expenditure, such as respirometry or doubly labeled water, are well established (Lifson et al. 1955; Lighton 2008), obtaining direct measurements of energy acquisition (i.e., food intake) in free-ranging organisms can be more challenging (Humphries et al. 2003). Behavioral studies (e.g., focal observations) are an effective way to quantify energy acquisition for some species (e.g., Humphries et al. 2002), but observing many species in the act of feeding is difficult to impossible, particularly for small-bodied or cryptic animals. Another technique is to record changes in body mass over subsequent captures, but for many species the low likelihood of recapture precludes this approach. Alternative methods are therefore necessary to quantify energy acquisition in free-living animals.

Plasma metabolite analysis provides an opportunity to estimate patterns of energy acquisition without relying on direct observation (Jenni-Eiermann and Jenni 1994; Mellish et al. 2001; Zajac et al. 2006; McGuire et al. 2009*a*; Kelm et al. 2011; Price et al. 2012). Plasma metabolite analysis has been used to assess stopover refueling rates (Schaub and Jenni 2001; Guglielmo et al. 2005; Zajac

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et al. 2006), foraging habitat quality (Guglielmo et al. 2005; Williams et al. 2007; Cravens and Boyles 2019), and demographic and population differences in fueling rates (McGuire et al. 2009*b*, 2016; Boyles et al. 2016), as well as to compare strategies for energy balance of migratory versus nonmigratory individuals (Boyle et al. 2010). These studies quantified concentrations of one or more metabolites, including β-hydroxybutyrate or triglycerides, both of which reflect changes in metabolic state as a function of fuel intake, assimilation, and mobilization. Dietary triglycerides are digested, absorbed in the intestine, and stored in adipocytes (Lehner and Kuksis 1996; Mead et al. 2002) for future liberation and use as metabolic fuel. Other macronutrients, including lipids, carbohydrates, and proteins, can be converted into triglycerides in the liver, which can then be found in circulation en route to storage in adipocytes or for use by tissues (Cryer 1980; Mead et al. 2002). Thus, triglyceride concentrations increase following feeding, regardless of the composition of the diet, and can be considered a feeding indicator. Moreover, metabolites provide relative indications of feeding rate. In a feeding trial experiment, switching Wilson's warblers (*Cardellina pusilla*) from a low to a high feeding rate resulted in increased plasma triglyceride concentration, with the increase observed within 10 min of the change in feeding rate (Zajac et al. 2006).

Stored nutrients provide energy during periods of fasting, and because carbohydrate stores are limited, fat is the primary fuel for most tissues during fasting. However, fatty acids cannot cross the blood-brain barrier, and therefore a constant supply of glucose is required to support the high metabolic costs of the brain (Raichle and Gusnard 2002). Ketone bodies, such as β-hydroxybutyrate, can be used as an alternative fuel source by the brain and other tissues and are typically found in high concentration in circulation during fasting (Robinson and Williamson 1980; Zajac et al. 2006). In the Wilson's warbler feeding experiment described above, there was an inverse relationship between triglyceride and β-hydroxybutyrate concentration, with β-hydroxybutyrate concentration decreasing with increased feeding rate (Zajac et al. 2006). Therefore, in most animals assessed to date plasma triglyceride concentration serves as a feeding indicator and plasma β-hydroxybutyrate serves as a fasting indicator. Together, predictable profiles of these plasma metabolites can be used to assess the nutritional status of animals captured once, without the need for direct observation of foraging or subsequent recapture.

Birds are among the best-studied taxa in the context of plasma metabolites and energy assimilation (Jenni-Eiermann and Jenni 1994; Schaub and Jenni 2001; Guglielmo et al. 2002, 2005; Seaman et al. 2005, 2006; Cerasale and Guglielmo 2006*a*, 2006*b*; Zajac et al. 2006; Anteau and Afton 2008). Despite similarities between temperate-zone birds and bats, few studies have quantified energy acquisition for bats (but see McGuire et al. 2009*a*, 2009*b*; Boyles et al. 2016; McGuire et al. 2016). Understanding energy acquisition and assimilation for temperate-zone bats is critical because they regularly face situations of extreme energetic demand and/or low energy availability (e.g., pregnancy and lactation, migration, hibernation). Some temperate-zone bats, including little brown bats (*Myotis lucifugus*), may hibernate for >240 d (Norquay and Willis 2014), and prehibernation fat stores appear to influence both within-hibernation energetics and emergence phenology (Jonasson and Willis 2012; Norquay and Willis 2014; Czenze and Willis 2015; Czenze et al. 2017). Fat storage in autumn likely influences future survival and reproduction and may be especially important for winter survival from white-nose syndrome (WNS), a recently emerged fungal disease devastating populations of little brown bats and other hibernating species across eastern North America (Frick et al. 2017). Among other pathophysiology, WNS causes increased frequency of arousals and torpid metabolic rate during hibernation, dramatically increasing energy expenditure and the depletion of fat stores (Reeder et al. 2012; Warnecke et al. 2012; McGuire et al. 2017), and recent evidence suggests that the size of the fall fat store is associated with persistence of bats after WNS invasion in some (though not all) hibernation sites (Cheng et al. 2019). Thus, understanding fall energy acquisition in bat species affected by WNS could be critical for identifying traits important for survival in remnant populations.

The use of plasma metabolite analysis has been validated for insectivorous bats (McGuire et al. 2009*a*), but the pattern of metabolite concentration changes in response to feeding is not the same as observed in other taxa. In other groups, triglyceride concentration increases following feeding while β-hydroxybutyrate concentration increases with fasting (Jenni-Eiermann and Jenni 1994; Guglielmo et al. 2002, 2005; Zajac et al. 2006). However, insectivorous bats increase both triglyceride and β-hydroxybutyrate concentrations after foraging (McGuire et al. 2009*a*; Boyles et al. 2016). When validating the technique, McGuire et al. (2009*a*) suggested that the unexpected β-hydroxybutyrate response may be a consequence of the energetic costs of flight for aerial-hawking bats. Aerial hawking is more energetically expensive than the foraging strategies of previously studied birds, which typically obtain their food from gleaning or surface feeding. Bats may not be able to sustain aerial hawking without mobilizing stored energy as typically observed in fasting periods (McGuire et al. 2009*a*).

We tested a prediction of what we term the "fasting while foraging" hypothesis by quantifying plasma triglyceride and β-hydroxybutyrate concentrations following feeding in a manipulative experimental design that prevented flight. If feeding-related increases in β-hydroxybutyrate previously observed for insectivorous bats (McGuire et al. 2009*a*; Boyles et al. 2016) were related to the high cost of foraging flight, then we predicted that bats prevented from flying during feeding would exhibit patterns of triglycerides and β-hydroxybutyrate, typical of other taxa following feeding. However, if the unexpected increase in β-hydroxybutyrate concentration previously observed for fed bats reflects some mechanism other than flight, we predicted similar relationships for plasma β-hydroxybutyrate and triglycerides as observed by McGuire et al. (2009*a*, 2009*b*) and Boyles et al. (2016).

Methods

Development of Predictive Framework

Variation in plasma triglyceride concentration is well established as a feeding indicator (e.g., Jenni-Eiermann and Jenni 1994; Guglielmo et al. 2005; Zajac et al. 2006; McGuire et al. 2009*a*; Price et al. 2012). Therefore, we developed our predictions by considering patterns of β-hydroxybutyrate concentration relative to pat-

terns of triglyceride variation and designed a manipulative experiment to avoid the difficulty of interpreting a correlative study. In the wild, insectivorous bats typically have access to abundant prey resources and extended periods of nocturnal activity over which to pursue and consume prey and as a result may often feed to satiation one or more times per night (Anthony and Kunz 1977; Anthony et al. 1981). In other words, for free-living insectivorous bats in many contexts, neither food availability nor foraging time is likely a limiting resource. Both the amount of food eaten and the time since feeding affect metabolite concentrations, so in theory both could be experimentally manipulated to test the effects of flight on the relationship between feeding state and metabolite concentrations. In practice, however, the best experimental manipulation, to reveal the largest possible variation in metabolite concentrations, would be to adjust food availability but hold the time since feeding constant. The opposite approach is not feasible because of the functional limitations of consuming unlimited food in a limited time period. We provided a limited, fixed amount of food to bats housed in individual cages that prevented the bats from flying. We expected that bats would consume all the available food quickly, after which no additional food was provided. We sampled bats at varying time intervals after feeding, meaning the amount of time since the start of feeding continued to increase but the amount of food was fixed. Most of the variation in plasma metabolite concentrations that we observed will have reflected variation in the duration of trials from the time bats ingested food until their blood was sampled (i.e., 2–55 min) because most bats consumed almost all of their food in the first few minutes of their trials (fig. 2). However, there was between-individual variation in the timing of food intake, as well as the total mass of food consumed, and it was not possible to tease out the influence of these two effects or treat them as independent predictor variables in our analysis. Therefore, we used a ratio of the mass of food eaten (g) to the duration of the feeding trial until blood sampling (hereafter, intake rate) as our predictor variable. This variable captures the effect of time to blood sampling on plasma metabolite concentrations while also accounting for variation in the timing of food intake by different individuals. Therefore, we predicted that plasma triglyceride concentration would decline as intake rate declined. The fasting while foraging hypothesis predicts a negative relationship between intake rate and β-hydroxybutyrate if bats are permitted to fly (fig. 1*A*; McGuire et al. 2009*a*, 2009*b*; Boyles et al. 2016), but we designed the experiment to remove effects of flight on the relationship between intake rate and β-hydroxybutyrate. Therefore, if the energetic costs of aerial-hawking flight require the use of stored fat for fuel, then in the absence of flight, plasma β-hydroxybutyrate should act as a fasting indicator and show patterns consistent with those of other taxa (fig. 1*B*). However, if some other mechanism explains different patterns of β-hydroxybutyrate concentration for insectivorous bats, then preventing flight during feeding should not influence β-hydroxybutyrate concentration (fig. 1*C*). We recognize that our approach is limited in that we were not able to directly test for effects of flight on metabolite concentrations by flying bats. However, our experiment did allow us to evaluate one clear prediction of the fasting while foraging hypothesis.

Experimental Methods

We captured little brown bats from August 5 to September 7, 2014, using a harp trap placed at the entrance of St. George Bat Cave, a hibernaculum located near Fisher River, Manitoba (51°43′N, 97°24′W), and immediately released all subadults (identified based on the epiphyseal ossification of the second metacarpal joint on the fourth digit; Kunz and Anthony 1982). On capture, we transported adult bats ($n = 26$) ~20 km to a field laboratory in individual disposable paper bags hung from string inside a ventilated picnic cooler to dampen noise. We recorded sex, measured forearm length (mm), and weighed each bat on an electric balance (± 0.1 g; model HH 320; Ohaus, Parsippany, NJ).

We housed bats individually in stainless steel cages (20 cm × 20 cm × 20 cm) for up to 5 d to prevent bats from flying and to eliminate the influence of exercise on plasma metabolite con-

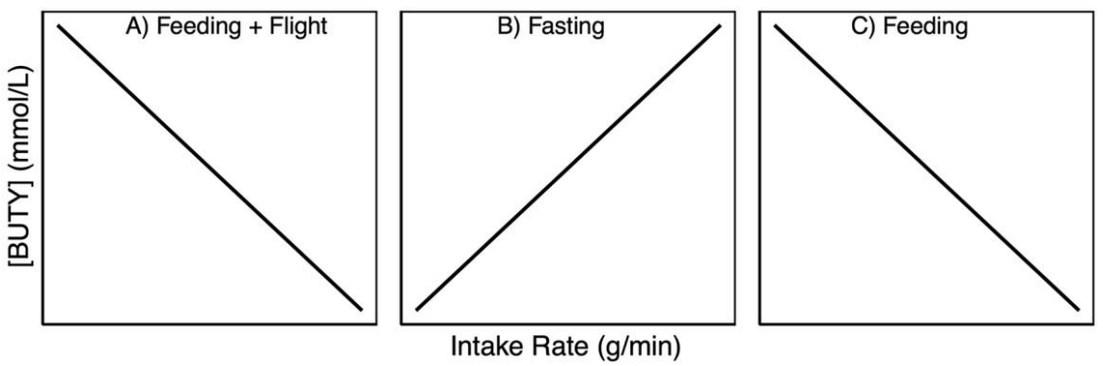

Figure 1. Hypothetical predictive plots of the effect of feeding state and flight on the relationship between intake rate and β-hydroxybutyrate concentration (BUTY; mmol/L). *A*, The predicted relationship between intake rate and β-hydroxybutyrate based on β-hydroxybutyrate concentrations observed in previous studies of wild-caught bats feeding on the wing. *B*, The predicted relationship of β-hydroxybutyrate concentration responding as a feeding indicator (negative relationship). *C*, The predicted relationship of β-hydroxybutyrate concentration responding as a fasting indicator (positive relationship).

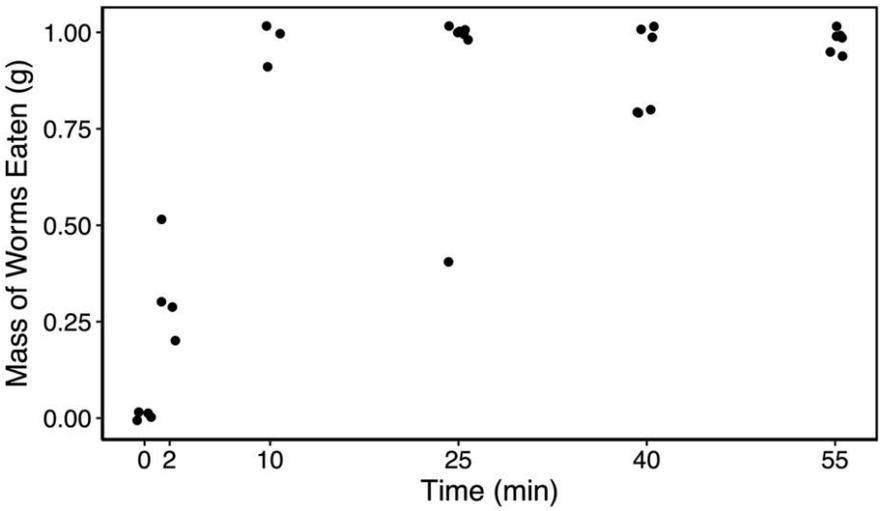

Figure 2. Mass of mealworms (*Tenebrio molitor* larvae; g) eaten by little brown bats (*Myotis lucifugus*) over time. Bats were presented with 1.0 g of food for a randomly assigned 0, 2, 10, 25, 40, or 55 min ($n = 4, 4, 3, 7, 6,$ or 6, respectively). Data points are set to deviate from the true value (vertically: ± 0.01 mmol/L; horizontally: ± 1.0 min) to show the number of bats at each time point.

centrations. We provided water ad lib. and trained bats to independently feed on mealworms (*Tenebrio molitor* larvae; Super Cricket, Prince Albert, Saskatchewan: http://www.supercricket.ca), a process that took 1–3 nights per bat. Composition of mealworms is typically 55% protein, 34% lipid, and 2% carbohydrate by dry mass (Super Cricket). Bats that failed to feed independently within 36 h of capture were released at the site of capture, leaving a total sample size of 26 bats.

After bats were trained to eat mealworms from food dishes, we began a series of feeding trials. On the night of testing, we placed a dish containing 1.0 g of mealworms (approximately 10 mealworms) in each cage. We observed the bat to determine the time when feeding began, which became the start of the "treatment group" time. Due to their small body size, blood could be collected from each bat only once, so we randomly assigned each bat to one of five treatment groups for which blood was sampled at either 2, 10, 25, 40, or 55 min after feeding ($n = 4, 3, 7, 6,$ or 6, respectively). We selected these time points based on the response times for a similarly sized passerine bird (Zajac et al. 2006). During the experiment, bats were left undisturbed until they were removed from their cage for sampling. After collecting a blood sample (see below), we recorded the remaining mass of uneaten mealworms (if any).

To collect blood, we gently restrained bats on a disinfected table with the tail membrane extended. We used a 27-gauge syringe needle (Becton Dickinson, Mississauga, Ontario) to puncture the interfemoral vein and collected up to 70 μL of blood from an individual bat using a heparinized capillary tube (Fisher Scientific, Pittsburgh). We sealed capillary tubes with critoseal (McCormick Scientific, St. Louis), spun them in a hematocrit centrifuge for 5 min at 10,000 rpm, transferred plasma to a 1-mL O-ring sealed cryotube (Fisherbrand, Pittsburgh), and stored the samples in a -20°C freezer for up to 7 d until we could transport them to a -80°C freezer. Stress can influence plasma metabolites, so we recorded handling time for each bat starting immediately after the bat was removed from its cage and ending either when 70 μL of blood was sampled or after 10 min (Jenni-Eiermann and Jenni 1996, 1997; Cyr et al. 2007).

We quantified triglyceride and β-hydroxybutyrate concentrations following manufacturer protocols modified for small sample volumes (5 μL plasma) in 96-well microplates as described by Guglielmo et al. (2002, 2005) and used by McGuire et al. (2009*a*, 2009*b*). We analyzed undiluted plasma following methods in previous studies of insectivorous bats (McGuire et al. 2009*a*, 2009*b*). We measured plasma triglyceride concentration by subtracting free glycerol from total glycerol following lipoprotein lipase hydrolysis (Sigma-Aldrich, Oakville, Ontario; Free Glycerol Reagent, Sigma-Aldrich, St. Louis). We measured β-hydroxybutyrate concentration using a commercial kinetic assay kit (R-Biopharm, Marshall, MI). Both assays were conducted using a microplate spectrophotometer (SpectraMax i3; Molecular Devices, Sunnyvale, CA). All samples were analyzed in duplicate, and we used mean values of the duplicate samples for subsequent analysis. If the coefficient of variation (CV) between measurements was >15% an additional replicate was included and the two replicates with the lowest CV were taken for analysis. Sample volume was limited for many individuals, and if it was not possible to obtain a CV $< 15\%$, the sample was excluded from statistical analyses.

We conducted all analyses using R (ver. 3.4.4; R Development Core Team 2018). Due to sample size, we pooled data from males and females. Before analyses, we assessed normality and collinearity among covariates. We found no effects of Julian date or ambient temperature at the time of blood sampling on the concentration of metabolites, so both were excluded from subsequent analyses. To test for differences in the predicted responses of triglyceride and β-hydroxybutyrate concentrations, we used an ANCOVA with intake rate and plasma metabolite ID (i.e., either triglyceride or β-hydroxybutyrate) as predictor variables, includ-

ing the interaction between plasma metabolite ID and intake rate. We used the drop1 function to calculate statistics for our model using type III sum of squares.

All methods were approved by the University of Winnipeg Animal Care Committee and conducted under Manitoba Conservation Wildlife Scientific permit AE03395. Although our study site was negative for *Pseudogymnoascus destructans*, the fungal pathogen that causes WNS, we followed established guidelines for decontamination by researchers (United States Fish and Wildlife Service 2015; Canadian Wildlife Health Cooperative 2015).

Results

A plot of the relationship between the mass of food eaten and trial duration suggests that, as expected, most bats ate the mealworms quickly (fig. 2). With the exception of one individual, all bats sampled at 10 min or later had eaten all of the food provided. Therefore, the bats' metabolite concentrations should reflect rapid food consumption, followed by roosting for the duration of their trial, validating our prediction of a negative relationship between intake rate and concentration of triglyceride.

We obtained metabolite concentrations from 26 individual bats. Mean bleed time was 6.9 $\pm$ 2.4 min (range: 1.4–9.2 min). Mean mass of food eaten was 0.84 $\pm$ 0.3 g (range: 0.1–1.0 g; fig. 2), leading to a mean intake rate of 0.06 $\pm$ 0.05 g min^{-1}. Mean body mass was 7.8 $\pm$ 0.7 g. Our field site experienced typical ambient temperatures during feeding trials that ranged from 9.0° to 22.5°C.

As predicted for our feeding indicator, we observed a negative relationship between intake rate and plasma triglyceride concentration ($F_{1, 33} = 8.3, P = 0.007$; fig. 3). Contrary to the prediction of the fasting while foraging hypothesis, however, we observed a negative relationship between intake rate and β-hydroxybutyrate concentration, with greater β-hydroxybutyrate concentrations compared to triglyceride ($F_{1, 34} = 57.0, P < 0.0001$; fig. 3). We did not detect an interaction between intake rate and metabolite ID ($F_{1, 34} = 0.18, P = 0.67$), indicating no difference in the slopes of the relationships for triglyceride and β-hydroxybutyrate.

Discussion

Our results demonstrate that intake rate affects the concentrations of plasma metabolites in the circulation of insectivorous bats, but we found no support for our fasting while foraging hypothesis. Specifically, we found a negative linear relationship between intake rate and plasma triglyceride concentrations, confirming that triglyceride concentrations respond to feeding in insectivorous bats. Contrary to our expectation, however, we found a negative linear relationship between intake rate and plasma β-hydroxybutyrate concentrations that was similar to the relationship for triglycerides. The similarity in responses of triglyceride and β-hydroxybutyrate suggests that flight alone does not affect the concentration of β-hydroxybutyrate in circulation.

McGuire et al. (2009*a*) proposed the hypothesis that energy expenditure resulting from an aerial-hawking foraging strategy required an increase in circulating fuel. Consistent with all previous studies, triglyceride concentrations responded as predicted to feeding in our bats. However, despite having removed the influence of flight, we observed similar patterns for triglyceride and β-hydroxybutyrate, consistent with previous studies of bats

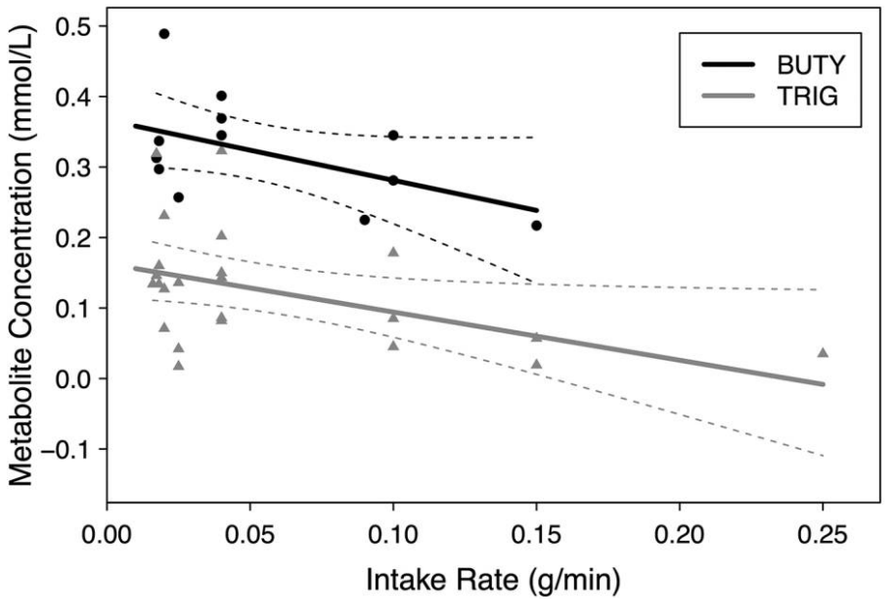

Figure 3. Negative relationships between intake rate and plasma β-hydroxybutyrate and triglyceride concentrations (mmol/L) of recently fed little brown bats (*Myotis lucifugus*). Intake rate is calculated using the total mass of food eaten (capped at 1.0 g of mealworms; see "Methods" for more details) during a trial or at either 0, 2, 10, 25, 40, or 55 min. Each bat experienced only one trial. Plasma β-hydroxybutyrate concentration is shown in black (BUTY; $y = -0.67x + 0.36$), while triglyceride is shown in gray (TRIG; $y = -0.67x + 0.17$). Black and gray dashed lines indicate 95% confidence intervals around their respective metabolite trend lines.

but counter to studies in other taxa. The mechanism underlying this pattern remains unclear. It could be that the increase in β-hydroxybutyrate we observed reflects an adaptation for preserving glucose for consumption by the brain regardless of activity state. Gao et al. (2009) found that "obese strain" mice fed butyrate had lower levels of insulin, which would have suppressed uptake of glucose from circulation, and increased adaptive oxidation of fatty acids. Not only is it necessary to preserve glucose when low dietary glucose is available, but suppressing uptake of glucose could also prioritize the use of fatty acids to fuel flight. One possibility is that species with relatively low levels of carbohydrates in their diet require relatively high β-hydroxybutyrate levels to ensure fuel supply to brain tissue, while exhibiting a greater tendency to use fatty acids as fuel for energetically expensive activity.

To better understand the response of β-hydroxybutyrate concentration to feeding and flight, we recommend additional studies quantifying physiological responses of swifts or swallows to recent feeding. Swifts and swallows experience energetic costs similar to those of insect-eating bats, as they use a similar aerial-hawking foraging strategy and likely experience similar energetic demands while foraging. Responses in these birds would allow us to determine whether the unusual response of β-hydroxybutyrate to feeding for aerial-hawking, insect-eating bats reflects physiological demands of an aerial-hawking foraging strategy or some mechanistic difference between birds and bats. Future studies should also quantify the effect of flight without feeding on plasma metabolites. Although we have shown that β-hydroxybutyrate concentrations respond to feeding without flight, we cannot yet say that flight does not also elicit some additional response in fuel production and/or circulation. Recently, Cravens and Boyles (2019) suggested the possibility that a separate molecular pathway exists for producing and circulating fuels when individuals have fed and are postabsorptive and before exogenous food is digested to ensure adequate fuel is available to power foraging flight.

A growing number of studies have quantified plasma metabolite concentrations, and it is tempting to compare values across studies for context. However, values from our study are likely not directly comparable to other values published for free-ranging bats. The relatively high triglyceride concentrations reported for bats returning to their roosts after foraging in past studies (e.g., McGuire et al. 2009*b* [0.44 ± 0.4 mmol/L] vs. this study [0.12 ± 0.08 mmol/L]) could reflect multiple or extended foraging bouts by free-ranging bats that occurred throughout the latter part of the night, as opposed to the single feeding event for bats in our study. Foraging rates of free-ranging juvenile (1.8 g/day) and pregnant (2.5 g/day) *Myotis lucifugus* are considerably larger than the 0.84 ± 0.3 g food consumed by our bats, and as such, concentrations of plasma metabolites that we observed were relatively low. Each bat in our study did, however, eat ~9.5% of their body mass before metabolite sampling, and all bats maintained body mass during their time in captivity, which suggests that the food volumes in our experiment were ecologically relevant if lower than natural feeding volumes. Despite the fact that bats in our study ate less food than typically consumed by free-ranging bats over the course of a night, our results still show that the changes in concentrations of metabolites (i.e., β-hydroxybutyrate and triglyceride) are consistent with other studies (other mammals: Galster and Morrison 1975; Arnould et al. 2001; other insect-eating bats: Widmaier et al. 1996; McGuire et al. 2009*a*, 2009*b*).

Another potential issue for our study was the potential for capture stress to influence our results because plasma metabolite concentrations are affected by acute stress response (Jenni-Eiermann and Jenni 1996, 1997; Cyr et al. 2007). We minimized the possibility of stress effects by holding bats in captivity for a minimum of 3 d before measurement and by not handling bats within 24 h of their assigned feeding trials. Little is known about the effects of chronic stress on metabolite concentrations, although captivity can increase chronic stress. We assume bats experienced little chronic stress because all individuals readily ate the mealworms we provided, maintained body mass, and appeared healthy throughout captivity.

North American bats, including our study species, currently face an ecological crisis from WNS, a disease that fundamentally alters energy balance during hibernation (Reeder et al. 2012; Warnecke et al. 2012; Verant et al. 2014; McGuire et al. 2017). Since the discovery of WNS in 2006, 11 hibernating species have been confirmed with the disease, including three species now listed as endangered in Canada because of the disease (COSEWIC 2015). Understanding pre- and posthibernation energetics of WNS-impacted bat species has become crucially important for conservation and management, and profiles of plasma metabolites, such as those presented in this study, in combination with increasingly accessible handheld analytical tools (Boyles et al. 2016; Sommers et al. 2017), could be important for understanding feeding energetics of free-living bats in the wild. For example, indexes of food intake of wild-captured bats could be effective for understanding the influence of variation in habitat quality for remnant bat populations and for evaluating potential management actions to improve habitats in ways that enhance energy balance of free-ranging bats (Langwig et al. 2016).

Our study is among the first to compare feeding rate to plasma metabolite responses of insect-eating bats, which provides insight that could be useful for understanding energy acquisition and assimilation in endangered species, including little brown bats. Plasma metabolite analyses are becoming more accessible and efficient ways of studying energetics of free-living bats in the field. As these field-ready physiological techniques improve, we are also gaining a better understanding of how bats interact with their habitats and finding continued support that metabolite analyses are an accessible and minimally invasive method for determining the feeding state of wildlife.

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Literature Cited

Anteau M.J. and A.D. Afton. 2008. Using plasma-lipid metabolites to index changes in lipid reserved of free-living lesser scaup (*Aythya affinis*). Auk 125:354–357.

Anthony E.L.P. and T.H. Kunz. 1977. Feeding strategies of the little brown bat, *Myotis lucifugus*, in southern New Hampshire. Ecology 58:775–786.

Anthony E.L.P., M.H. Stack, and T.H. Kunz. 1981. Night roosting and the nocturnal time budget of the little brown bat, *Myotis lucifugus*: effects of reproductive status, prey density, and environmental conditions. Oecologia 51:151–156.

Arnould J.P.Y., J.A. Green, and D.R. Rawlins. 2001. Fasting metabolism in Antarctic fur seal (*Arctocephalus gazelle*) pups. Comp Biochem Physiol A 129:829–841.

Bailey H., S. Fossette, S.J. Bograd, G.L. Shillinger, A.M. Swithenbank, J.-Y. Georges, P. Gaspar, et al. 2012. Movement patterns for critically endangered species, the leatherback turtle (*Dermochelys coriacea*), linked to foraging success and population status. PLoS ONE 7:e36401.

Boyle W.A., D.R. Norris, and C.G. Guglielmo. 2010. Storms drive altitudinal migration in a tropical bird. Proc R Soc B 277:2511–2519.

Boyles J.G., L.P. McGuire, E. Boyles, J.P. Reime, C.A.C. Brooks, R.W. Rutherford, T.A. Rutherford, J.O. Whitaker Jr., and G.F. McCracken. 2016. Physiological and behavioural adaptations in bats living at high latitudes. Physiol Behav 165:322–327.

Canadian Wildlife Health Cooperative. 2015. Canadian national white-nose syndrome decontamination protocol for entering bat hibernacula. Canadian Wildlife Health Cooperative, Saskatoon.

Cerasale D.J. and C.G. Guglielmo. 2006*a*. Dietary effects on prediction of body mass changes in birds by plasma metabolites. Auk 123:836–846.

———. 2006*b*. Plasma metabolite profiles: effect of dietary phospholipids in a migratory passerine (*Zonotrichia leucophrys gambelii*). Physiol Biochem Zool 79:754–762.

Cheng T.L., A. Gerson, M.S. Moore, J.D. Reichard, J. DeSimone, C.K.R. Willis, W.F. Frick, and A.M. Kilpatrick. 2019. Higher fat stores contribute to persistence of little brown bat populations with white-nose syndrome. J Anim Ecol 88:591–600.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2015. Committee on the Status of Endangered Wildlife in Canada. https://www.canada.ca/en/environment-climate-change/services/committee-status-endangered-wildlife.html.

Cravens Z.M. and J.G. Boyles. 2019. Illuminating the physiological implications of artificial light on an insectivorous bat community. Oecologia 189:69–77.

Cryer A. 1980. Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. Int J Biochem 13:525–541.

Cyr N.E., K. Earle, C. Tam, and L.M. Romero. 2007. The effect of chronic psychological stress on corticosterone, plasma metabolites, and immune responsiveness in European starlings. Gen Comp Endocrinol 154:59–66.

Czenze Z.C., K.A. Jonasson, and C.K.R. Willis. 2017. Thrifty females, frisky males: winter energetics of bats from a cold climate. Physiol Biochem Zool 90:502–511.

Czenze Z.C. and C.K.R. Willis. 2015. Warming up and shipping out: cues for arousal and emergence in hibernating little brown bats (*Myotis lucifugus*). J Comp Physiol B 185:575–586.

Frick W.F., T.L. Cheng, K.E. Langwig, J.R. Hoyt, A.F. Janicki, K.L. Parise, J.T. Foster, and A.M. Kilpatrick. 2017. Pathogen dynamics during invasion and establishment of white-nose syndrome explain mechanisms of host persistence. Ecology 98:524–631.

Galster W. and P.R. Morrison. 1975. Gluconeogenesis in arctic ground squirrels between periods of hibernation. Am J Physiol 288:325–330.

Gao Z., J. Yin, J. Zhang, R.E. Ward, R.J. Martin, M. Lefevre, W.T. Cefalu, and J. Ye. 2009. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes 58:1509–1517. doi:10.2337/db08-1637.

Guglielmo C.G., D.J. Cerasale, and C. Eldermire. 2005. A field validation of plasma metabolite profiling to assess refueling performance of migratory birds. Physiol Biochem Zool 78:116–125.

Guglielmo C.G., P.D. O'Hara, and T.D. Williams. 2002. Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living western sandpipers (*Calidris mauri*). Auk 119:437–445.

Humphries M.M., D.L. Kramer, and D.W. Thomas. 2003. The role of energy availability in mammalian hibernation: an experimental test in free-ranging eastern chipmunks. Physiol Biochem Zool 76:180–186.

Humphries M.M., D.W. Thomas, C.L. Hall, J.R. Speakman, and D.L. Kramer. 2002. The energetics of autumn mast hoarding in eastern chipmunks. Oecologia 133:30–37.

Jenni-Eiermann S. and L. Jenni. 1994. Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the garden warbler. Auk 111:888–899.

———. 1996. Metabolic differences between the postbreeding, moulting and migratory periods in feeding and fasting passerine birds. Funct Ecol 10:62–72.

———. 1997. Diurnal variation of metabolic responses to short-term fasting in passerine birds during the postbreeding, molting and migratory period. Condor 99:113–122.

Jonasson K.A. and C.K.R. Willis. 2012. Hibernation energetics of little brown bats. J Exp Biol 215:2141–2149.

Kelm D.H., R. Simon, D. Kuhlow, C.C. Voigt, and M. Ristow. 2011. High activity enables life on a high-sugar diet: blood glucose regulation in nectar-feeding bats. Proc R Soc B 278:1–7.

Kronfeld-Schor N. and T. Dayan. 2013. Thermal ecology, environments, communities, and global expenditure in endotherms. Annu Rev Ecol Evol Syst 44:461–480.

Kunz T.H. and E.L.P. Anthony. 1982. Age estimation and postnatal growth in bat *Myotis lucifugus*. J Mammal 63:23–32.

Langwig K.E., J.R. Hoyt, K.L. Parise, W.F. Frick, J.T. Foster, and A.M. Kilpatrick. 2016. Resistance in persisting bat populations after white-nose syndrome invasion. Philos Trans R Soc B 372: 20160044.

Lehner R. and A. Kuksis. 1996. Biosynthesis of triacylglycerols. Progress in Lipid Research 35:169–201.

Lifson N., G.B. Gordon, and R. McClintock. 1955. Measurement of total carbon-dioxide production by means of D2O18. J Appl Physiol 7:704–710.

Lighton J.R.B. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, Oxford.

McGuire L.P., M.B. Fenton, P.A. Faure, and C.G. Guglielmo. 2009*a*. Determining feeding state and rate of mass change in insectivorous bats using plasma metabolite analysis. Physiol Biochem Zool 82:812–818.

McGuire L.P., M.B. Fenton, and C.G. Guglielmo. 2009*b*. Effect of age on energy storage during prehibernation swarming in little brown bats (*Myotis lucifugus*). Can J Zool 87:515–519.

McGuire L.P., H.W. Mayberry, and C.K.R. Willis. 2017. White-nose syndrome increase torpid metabolic rate and evaporative water loss in hibernating bats. Am J Physiol 313:R680–R686.

McGuire L.P., K. Muise, A. Shrivastav, and C.K.R. Willis. 2016. No evidence of hyperphagia during pre-hibernation in a northern population of little brown bats (*Myotis lucifugus*). Can J Zool 94:821–827.

Mead J.R., S.A. Irvine, and D.P. Ramji. 2002. Lipoprotein lipase: structure, function, regulation, and role in disease. J Mol Med 80:753–769.

Mellish J.E. and S.J. Iverson. 2001. Blood metabolites as indicators of nutrient utilization in fasting, lactating phocid seals: does depletion of nutrient reserves terminate lactation? Can J Zool 79:303–311.

Moeller K.T., M.W. Butler, and D.F. DeNardo. 2013. The effect of hydration state and energy balance on innate immunity of a desert reptile. Front Zool 10:1–10.

Norquay K.J.O. and C.K.R. Willis. 2014. Hibernation phenology of *Myotis lucifugus*. J Zool (Lond) 294:85–92.

Price E.R., T.T. Jones, B.P. Wallace, and C.G. Guglielmo. 2012. Serum triglycerides and β-hydroxybutyrate predict feeding status in green turtles (*Chelonia mydas*): evaluating a single blood sample method for assessing feeding/fasting in reptiles. Exp Mar Biol Ecol 439:176–180.

Raichle M.E. and D.A. Gusnard. 2002. Appraising the brain's energy budget. Proc Natl Acad Sci USA 99:10237–10239.

R Development Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

Reeder D.M., C.L. Frank, G.G. Turner, C.U. Meteyer, A. Kurta, E.R. Britske, M.E. Vodzak, et al. 2012. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. PLoS ONE 7:e38920. doi:10.1371/journal.pone.0038920.

Robinson A.M. and D.H. Williamson. 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol Rev 60:143–187.

Saino N., M. Romano, D. Rubolini, R. Ambrosini, A. Romano, M. Caprioli, A. Costanzo, and G. Bazzi. 2014. A trade-off between reproduction and feather growth in the barn swallow (*Hirundo rustica*). PLoS ONE 9:1–12.

Schaub M. and L. Jenni. 2001. Variation of fuelling rates among sites, days and individuals in migrating passerine birds. Funct Ecol 15:584–594.

Seaman D.A., C.G. Guglielmo, R.W. Elner, and T.D. Williams. 2006. Landscape scale physiology: site differences in refueling rates indicated by plasma metabolite analysis in free-living migratory sandpipers. Auk 123:563–574.

Seaman D.A., C.G. Guglielmo, and T.D. Williams. 2005. Effects of physiological state, mass change and diet on plasma metabolite profiles in the western sandpiper *Calidris mauri*. J Exp Biol 208:761–769.

Sommers A.S., W.A. Boyle, and L.P. McGuire. 2017. Validation of a field-ready meter for plasma β-hydroxybutyrate analysis. J Field Ornithol 88:399–404.

United States Fish and Wildlife Service. 2015. White-nose syndrome decontamination protocols. United States Government, Washington, DC.

Verant M.L., C.U. Meteyer, J.R. Speakman, P.M. Cryan, J.M. Lorch, and D.S. Blehert. 2014. White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. BMC Physiol 14:10.

Warnecke L., J.M. Turner, T.K. Bollinger, J.M. Lorch, V. Misra, P.M. Cryan, and C.K.R. Willis. 2012. Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. Proc Natl Acad Sci USA 109:6999–7003.

Widmaier E.P., E.R. Gornstein, J.L. Hennessey, J.M. Bloss, J.A. Greenberg, and T.H. Kunz. 1996. High plasma cholesterol, but low triglycerides and plaque-free arteries, in Mexican free-tailed bats. Am Physiol Soc 271:R1101–R1106.

Williams T.D., N. Warnock, J.Y. Takekawa, and M.A. Bishop. 2007. Flyway-scale variation in plasma triglycerides levels as an index of refueling rate in spring-migrating western sandpipers (*Calidris mauri*). Auk 124:886–897.

Womble J., G. Blundell, S. Gende, M. Horning, M. Sigler, and D. Csepp. 2014. Linking marine predator diving behaviour to local prey fields in contrasting habitats in a subarctic glacial fjord. Mar Biol 161:1361–1374.

Zajac R.M., D.J. Cersale, and C.G. Guglielmo. 2006. The rapid response of plasma metabolites to changes in feeding rates in a small passerine Wilson's warbler *Wilsonia pusilla*. J Avian Biol 37:405–408.