BIOLOGY LETTERS

royalsocietypublishing.org/journal/rsbl

Research

Cite this article: Rossi GS, Welch KC. 2024 Vampire bats rapidly fuel running with essential or non-essential amino acids from a blood meal. *Biol. Lett.* **20**: 20240453.
https://doi.org/10.1098/rsbl.2024.0453

Received: 6 August 2024
Accepted: 4 October 2024

Subject Category:
Physiology

Subject Areas:
evolution, ecology

Keywords:
vampire bat, blood, amino acid, fuel use, exercise

Author for correspondence:
Kenneth C. Welch
e-mail: kenneth.welchjr@utoronto.ca

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.7524683.

THE ROYAL SOCIETY PUBLISHING

Vampire bats rapidly fuel running with essential or non-essential amino acids from a blood meal

Giulia S. Rossi[1,2] and Kenneth C. Welch[1]

[1]Department of Biological Sciences, University of Toronto, Scarborough, Ontario M1C 1A4, Canada
[2]Department of Biology, McMaster University, Hamilton, Ontario L8S 4E8, Canada

GSR, 0000-0002-4812-8869; KCW, 0000-0002-3283-6510

In most mammals, running is fuelled by oxidization of endogenous carbohydrates and lipids while amino acids contribute little (< 5–10%). Common vampire bats (*Desmodus rotundus*), however, specialize on a unique, protein-rich blood diet. Therefore, we hypothesized that (i) vampire bats would rapidly begin utilizing dietary amino acids to support running metabolism, and (ii) that relative reliance on essential and non-essential amino acids would be similar. We fed bats cow's blood enriched either with isotopically labelled glycine (non-essential amino acid) or leucine (essential amino acid). Bats were exercised at speeds of 10, 20 and 30 m min^{-1} on a respirometry treadmill, allowing us to assess metabolic rate (i.e. O_2 consumption and CO_2 production) and track the oxidation of labelled amino acids in exhaled CO_2. Vampire bats oxidized amino acids as their primary fuel as indicated by a respiratory exchange ratio (RER = ratio of CO_2 production to O_2 consumption rates) of approximately 0.8–0.9 at all speeds, with the labelled meal accounting for as much as 60% of oxidized fuels at peak usage. Similar oxidation rates indicated bats did not discriminate between essential and non-essential amino acid use. These findings reiterate how strongly metabolism can be shaped by a specialized diet.

1. Introduction

Among mammals, it is well-established that low-intensity aerobic exercise is predominately fuelled by lipids, with a higher proportion of carbohydrates oxidized as exercise intensity increases (for reviews, see [1,2]). Amino acids tend to be overlooked as a metabolic fuel because their oxidation often contributes less than 5–10% of the total ATP production during exercise [3,4]. However, some mammals have diets extremely rich in protein but relatively poor in carbohydrates and lipids, including the three species of vampire bats that are the only mammalian obligate sanguinivores (*Desmodus rotundus*, *Diphylla ecudata* and *Diaemus youngi*).

While most mammals appear constrained to oxidize an exercise-intensity-dependent mixture of lipids and endogenous carbohydrates, the same is not true of other groups. Specifically, flying insects exhibit distinct patterns of fuel use during exercise (flight) generally correlated with differences in diet [5]. For example, most bees that subsist primarily on sugar-rich floral nectar rely exclusively on carbohydrate oxidation during flight [6]. In contrast, obligately haematophagous tsetse flies (*Glossina* spp.) rely on proline oxidation to sustain flight [7,8], while the facultatively haematophagous female *Aedes aegypti* mosquito, but not the strictly nectar or fruit-eating male mosquito, shows a similar ability [9]. The ability to rely on sugar rapidly and completely to fuel foraging flight appears to have convergently evolved in nectar-feeding insects [6], bats [10,11] and hummingbirds [12,13]. Among vertebrates,

phyllostomid bats exhibit remarkable dietary diversity. With this in mind, we asked if vampire bats had evolved a capacity for high rates of amino acid oxidation during exercise similar to that of an invertebrate group upon which they have converged in diet, obligately haematophagous insects.

Unlike most other bats, vampire bats exhibit exceptional running ability, allowing them to track and approach their prey along the ground [14,15]. This unique running ability provides a tractable modality by which to interrogate protein use during exercise, because rather than flying the bats, we can capitalize on respiratory treadmill equipment that is otherwise used for studying rodents (figure 1). We first tested the hypothesis that vampire bats would exhibit rapid and extensive use of ingested amino acids to fuel terrestrial locomotion given their protein-rich diet. We provided isotopically labelled amino acids (leucine or glycine) in a cow blood meal to common vampire bats (*D. rotundus*) and then measured their metabolic rate (O_2 consumption and CO_2 production) at treadmill speeds of 10, 20 and 30 m min^{-1}. At the lowest (10 m min^{-1}) and highest (30 m min^{-1}) treadmill speeds, we obtained breath samples to detect the ^{13}C-enriched isotopic signature in exhaled CO_2, indicating whether the enriched amino acid tracers were being used to fuel exercise metabolism. Given how the vampire bat diet is rich in both essential (e.g. leucine) and non-essential (e.g. glycine) amino acids, we also questioned whether the use of the two classes of amino acids would be similar.

2. Material and methods

(a) Experimental animals and study site

We captured 24 adult vampire bats (*D. rotundus*) between 25 April and 5 May 2023 in the tropical forests at the Lamanai Archeological Reserve in Lamanai, Orange Walk District, Belize (10 females, 14 males; table 1). All bats were captured using either mist nets or harp traps that were set across known flight paths shortly after sunset (approximately 18.15). Shortly after capture, bats were individually placed in drawstring cloth bags to prevent escape and minimize stress. We maintained bats in their cloth bags for up to 18 h after capture, which helped to ensure that bats were in a post-absorptive state prior to re-feeding for our experimental trials. The mean maximum and minimum daily temperatures during our study period were 34.3 and 22.0°C, respectively, and the average daily humidity was 65.9%. Our study period fell within the dry season and thus we only received 0.1 cm of rainfall. All climate data were retrieved from the National Meteorological Service of Belize for Tower Hill in Orange Walk District (http://nms.gov.bz). We weighed (30.3 ± 1.4 g) and released all bats back to their place of capture when the experiment concluded.

(b) Experimental groups

To understand whether *D. rotundus* use recently ingested blood meals to fuel aerobic exercise, we fed each bat cow's blood that was either enriched with isotopically labelled leucine (n = 9) or glycine (n = 12) at concentrations of approximately 2–3 mg ml^{-1}. An additional group of bats were fed unenriched cow's blood to serve as a control group (n = 3), providing baseline $\delta^{13}C$ signatures for bat breath during each stage of the experiment (e.g. across varying treadmill speeds). Cow blood was obtained from a local slaughterhouse in Orange Walk District, Belize, and treated with sodium citrate (2.75 g l^{-1}) and citric acid (1 g l^{-1}) to prevent spoiling. We maintained the blood at 4°C throughout the study period. Immediately prior to experimentation, we fed bats by gently injecting blood into the mouth using a 1 ml transfer pipette. All bats consumed similar amounts of blood regardless of the amino acid treatment, and body mass was similar across all experimental groups (table 1; electronic supplementary material, figure S1).

(c) Metabolic treadmill protocol and analysis

After feeding, we placed bats into a respiratory treadmill (internal dimensions 66.0 cm (L) × 14.9 cm (W) × 12.7 cm (H)) that was custom-built by Sable Systems International for this experiment, modelled after the Promethion Core Mouse Respirometry Treadmill (Sable Systems International, North Las Vegas, NV). We continuously flushed the treadmill with ambient air at flow rate of approximately 13 l min^{-1}, which was monitored using a mass flow controller (Flowbar-8 Mass Flow Meter System; Sable Systems International). A subsample of approx. 350 ml min^{-1} was pulled from an excurrent port directly into a gas analyser (FMS Field Metabolic System; Sable Systems International) to obtain O_2, CO_2 and water vapour measurements every second.

After placing a bat in the treadmill (7.6 ± 0.8 min after feeding), we steadily increased the treadmill speed to 10 m min^{-1} in 1 m min^{-1} increments. We did not provide bats with an acclimation period to the treadmill chamber while the belt was stationary because in preliminary trials, bats would explore the treadmill chamber and learn the location of small crevasses in which they could hook their thumbs and/or toes to avoid contact with the moving belt. At 10 m min^{-1}, bats exhibited a walking gait that is similar to the lateral-sequence walking gait of other tetrapods, in which a hindlimb touches the ground, followed by the ipsilateral forelimb (figure 1*a*) [15]. Once bats demonstrated this steady walking gait for 1–2 min, we steadily increased the treadmill speed to 20 m min^{-1}, in which bats exhibited a combination of a walking and running gait. This intermediate gait consisted of a lateral-sequence walking gait with occasional hops, where hindlimbs touch the ground simultaneously, followed by the simultaneous movement of both forelimbs. Again, once bats demonstrated this intermediate walking–running gait for 1–2 min, we steadily increased the treadmill speed to 30 m min^{-1}. At 30 m min^{-1}, bats exhibited a steady running gait, in which hops occurred consistently, each with a notable aerial phase (figure 1*b*) [15]. After exhibiting 1–2 min of consistent running, we

Figure 1. Photographs of *Desmodus rotundus* (male; BZ 1256) on a metabolic treadmill in (*a*) a walking gait (10 m min^{-1}), and (*b*) the aerial phase of a running gait (30 m min^{-1}). Photographs by Price Sewell.

removed the bat from the treadmill to provide them with a brief rest and to flush the chamber with ambient air (~5 min). We repeated this treadmill protocol two additional times to obtain O_2 and CO_2 measurements at each treadmill speed (10, 20 and 30 m min^{-1}) in triplicate. The second and third replicates began 25.2 ± 1.2 and 41.7 ± 1.4 min after the initial cow blood feed, respectively.

From the raw O_2 and CO_2 measurements, we calculated rates of O_2 consumption (VO_2) and CO_2 production (VCO_2) for each treadmill speed using Expedata software (v. 1.8.4; Sable Systems International), correcting for temperature and water vapour. We then used the ratio between VCO_2 and VO_2 to determine the RER, as well as the cost of transport (CoT) across all treadmill speeds. Videos of bats at each treadmill speed are available in the electronic supplementary materials.

(d) Stable isotope protocol and analysis

When bats exhibited steady walking at 10 m min^{-1} and steady running at 30 m min^{-1} in each exercise replicate, we used an excurrent syringe port on the treadmill to withdraw 12 ml of air. The air sample was immediately injected into a 12 ml Exetainer vial (Labco, UK) for transport to the University of Toronto, Scarborough, Ontario, Canada, where we used a stable carbon isotope analyzer (Picarro G22201-i Isotopic Analyzer; Picarro Inc., Santa Clara, CA) to assess the $\delta^{13}C$ signature of exhaled CO_2 in the sample. Because the sampled air contained a mixture of breath and ambient CO_2, the isotopic signature of only exhaled breath ($\delta^{13}C_{breath}$) was calculated using the following equation [16]:

$$\delta^{13}C_{breath} = [\delta^{13}C_{sample} - \delta^{13}C_{ambiant^*}(f_a)]/(1 - f_a)$$

where $\delta^{13}C_{sample}$ is the isotopic signature of the air excurrent from the chamber, f_a is the fraction of CO_2 in the sample from ambient air and $\delta^{13}C_{ambient}$ is an estimate of the isotopic signature of ambient air, which was calculated as the slope resulting from a linear regression between the $\delta^{13}C_{sample}$ of control bats and the f_a. We then calculated the proportion of expired CO_2 supported by labelled amino acids (f_{exo}) using the following equation [16]:

$$f_{exo} = (\delta^{13}C_{breath} - \delta^{13}C_{control})/(\delta^{13}C_{blood} - \delta^{13}C_{control})$$

where $\delta^{13}C_{control}$ is the average $\delta^{13}C_{breath}$ of control bats across all treadmill speeds, and $\delta^{13}C_{blood}$ is the isotopic signature of the labelled cow blood. It was important to determine the $\delta^{13}C$ signature of the cow blood samples from each treatment group to correct for differences in amino acid concentrations that arose from using a 'field balance' rather than an analytical balance. Briefly, we loaded 10 µl blood samples into tin capsules (Costech Analytical Technologies Inc., Valencia, CA) and dried the

Table 1. Capture and experimental details for individual vampire bats (*Desmodus rotundus*) used in this study.

bat ID	capture date (mm/dd/yyyy)	GPS coordinates for capture location	capture method	dietary treatment	volume consumed (ml)	mass (g)	sex
BZ 2070	04/25/2023	17°45'48.8"N, 88°39'25.6"W	mist net	glycine	0.6	25.10	male
BZ 2186	04/25/2023	17°45'48.5"N, 88°39'21.5"W	harp trap	glycine	0.5	29.33	male
BZ 2090	04/25/2023	17°45'48.1"N, 88°39'26.8"W	mist net	glycine	0.3	24.12	male
BZ 2097	04/25/2023	17°45'49.1"N, 88°39'18.6"W	mist net	glycine	0.3	28.72	male
BZ 2403	04/28/2023	17°48'55.2"N, 88°43'50.0"W	harp trap	glycine	0.4	25.22	male
BZ 1034	04/29/2023	17°45′45.8"N, 88°39'25.7"W	harp trap	glycine	0.2	27.87	male
BZ 1801	04/29/2023	17°45′45.8"N, 88°39'25.7"W	harp trap	glycine	0.2	46.38	female
BZ 2461	04/30/2023	17°45'50.2"N, 88°39'13.4"W	harp trap	glycine	0.5	26.00	female
BZ 2463	04/30/2023	17°45'50.2"N, 88°39'13.4"W	harp trap	leucine	0.4	27.76	female
BZ 2467	04/30/2023	17°76'44.6"N, 88°65'34.9"W	harp trap	leucine	0.5	41.17	female
BZ 2462	04/30/2023	17°45'50.2"N, 88°39'13.4"W	harp trap	leucine	0.2	28.85	male
BZ 2546	05/04/2023	17°45'53.5"N, 88°39'07.1"W	mist net	leucine	0.6	25.68	male
BZ 2771	05/04/2023	17°45'53.5"N, 88°39'07.1"W	mist net	leucine	0.7	24.33	male
BZ 1256	05/04/2023	17°45'53.5"N, 88°39'07.1"W	mist net	leucine	0.4	29.06	male
BZ 2774	05/04/2023	17°45'53.5"N, 88°39'07.1"W	mist net	leucine	0.5	36.38	female
BZ 2775	05/04/2023	17°45'53.5"N, 88°39'07.1"W	mist net	leucine	0.4	36.80	female
BZ 2495	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	glycine	0.5	28.18	female
BZ 1724	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	glycine	0.4	37.16	male
BZ 1733	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	glycine	0.3	36.44	female
BZ 2829	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	glycine	0.5	24.24	female
BZ 2180	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	leucine	0.7	22.09	male
BZ 1027	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	control	0.4	27.16	male
BZ 0074	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	control	0.6	35.38	female
BZ 2090	05/05/2023	17°45'53.5"N, 88°39'07.1"W	mist net	control	0.5	21.92	male

samples at 105°C for 2 h. We then determined the $\delta^{13}C_{blood}$ by loading the tin capsules into the Picarro Combustion Module (Picarro Inc., Santa Clara, CA), which was connected to the Picarro G22201-i Isotopic Analyzer (Picarro Inc.).

(e) Statistical analysis

We analysed all data for statistical significance using RStudio (v. 1.1.463) with R (v. 3.6.1) and visualized the data using GraphPad Prism (v. 9.0.0). We used linear mixed effects models to analyse the effects of treadmill speed and dietary treatment on each metabolic trait (RER, VO_2, VCO_2, CoT), including body mass as a covariate and incorporating bat identity as a random intercept to account for repeated measurements on the same individual. We used a linear mixed effects model to analyse the effects of treadmill speed and dietary treatment on f_{exo}, again incorporating bat identity as a random intercept. In this model, we also included the exercise replicate as a fixed-effect, given that the oxidation of labelled amino acids may diminish with passing time. When significant main effects were detected, we used pairwise multiple comparison tests with a Bonferroni correction to determine differences between groups. Finally, we used one-way analyses of variance (ANOVAs) to demonstrate that blood consumption and body mass did not differ across the experimental treatment groups. All data were initially assessed for normality and homogeneity of variance. Results were considered significant at $\alpha = 0.05$.

3. Results

The RER is calculated as the ratio between CO_2 production and O_2 consumption (VCO_2/VO_2) and is routinely used as an indicator of metabolic fuel use, such that a ratio of 0.7 is indicative of fat oxidation, whereas a ratio of 1.0 indicates the exclusive use of carbohydrates. Running vampire bats exhibited a RER of 0.8–0.9, which remained consistent across all treadmill speeds ($t = 0.82$, $p = 0.42$; figure 2*a*). An RER of 0.8–0.9 can reflect a mixture of lipid and carbohydrate use, but may also indicate protein oxidation (for a review, see [17]). Although the RER remained consistent, we found that VCO_2 and VO_2 were significantly affected by exercise intensity (VCO_2: $t = 20.39$, $p < 0.001$; VO_2: $t = 16.04$, $p < 0.001$; figure 2*b*,*c*), both exhibiting a 15–20% increase with each 10 m min^{-1} rise in treadmill speed ($p < 0.001$). These findings indicate that vampire bats require more ATP to fuel exercise at higher intensities, which is similarly reflected by the increasing CoT at higher treadmill speeds ($t = 6.88$, $p < 0.001$; figure 2*d*). We found no effect of dietary treatment on the RER ($t = 0.08$, $p = 0.94$), VCO_2 ($t = 1.13$, $p = 0.27$), VO_2 ($t = 0.89$, $p = 0.38$), or CoT ($t = 0.43$, $p = 0.67$). CO_2 produced from oxidation of ingested amino acids was almost immediately present in the breath of exercising bats, suggesting the prompt use of the recent protein meal to fuel aerobic activity in this species. Indeed, we found that the time at which breath samples were collected significantly influenced the proportion of exhaled CO_2 exhibiting the ^{13}C isotopic signature of the tracer-enriched blood meal (i.e. f_{exo}; *sensu*[12]; $t = 9.85$, $p < 0.001$; figure 3). Reliance on the blood meal to fuel terrestrial locomotion occurred very rapidly after feeding, with f_{exo} exceeding 60% during the first exercise replicate on the treadmill. Over time, f_{exo} fell significantly as the absorbed substrates from the blood meal were either oxidized or otherwise sequestered from circulation into other tissues, reaching an average of 41 and 28% by the second and third exercise replicates, respectively ($p < 0.05$). We found no evidence indicating that vampire bats discriminate between the amino acids oxidized during exercise. The f_{exo} values were similar between bats fed cow's blood enriched with isotopically labelled leucine and glycine ($t = 1.02$, $p = 0.32$; figure 3).

4. Discussion

In support of our hypothesis, we found that vampire bats utilized amino acids as their primary fuel source during exercise. Bats exhibited a RER of 0.8–0.9, consistent with amino acid oxidation, which remained consistent across all treadmill speeds. Importantly, RER did not change despite varying exercise intensities (i.e. running speeds). If lipids and carbohydrates were the major metabolic substrates fueling exercise, we would have expected the RER to increase with increasing treadmill speed, reflecting the characteristic shift from lipid to carbohydrate oxidation at higher exercise intensities [18,19]. Although the RER remained consistent, we found that VCO_2 and VO_2 were significantly affected by exercise intensity, both exhibiting a 15–20% increase with each 10 m min^{-1} rise in treadmill speed. These findings confirm that, as predicted, vampire bats require more ATP to fuel exercise at higher intensities, which is similarly reflected by the increasing CoT at higher treadmill speeds.

CO_2 produced from oxidation of ingested amino acids was almost immediately present in the breath of exercising bats, suggesting the prompt use of the recent protein meal to fuel aerobic activity in this species. Indeed, we found that the time at which breath samples were collected significantly influenced the proportion of exhaled CO_2 exhibiting the ^{13}C isotopic signature of the tracer-enriched blood meal (i.e. f_{exo}; *sensu*[12]; figure 3). Reliance on the blood meal occurred very rapidly after feeding, with f_{exo} exceeding 60% during the first exercise replicated on the treadmill, which occurred within 10 min of feeding. Over time , f_{exo} fell significantly as the absorbed substrates were either oxidized or otherwise sequestered from circulation, reaching an average of 41 and 28% by the second and third exercise replicates, respectively. Importantly, the isotopically labelled amino acids in the blood meal may have been more readily available for oxidation compared to proteins, potentially leading to an overestimation of the reliance on a recent meal to fuel exercise. Indeed, Zhou *et al.* [20] found that mosquitos (*Aedes aegypti*) metabolized ^{14}C-labelled free amino acids in a protein-rich meal several hours before the significant hydrolysis of the meal protein occurred. However, vampire bats have evolved a unique gastrointestinal morphology and specialized mechanisms to expedite the digestion and assimilation of blood [21–24]. For instance, the gastric fundus of vampire bats exhibits higher

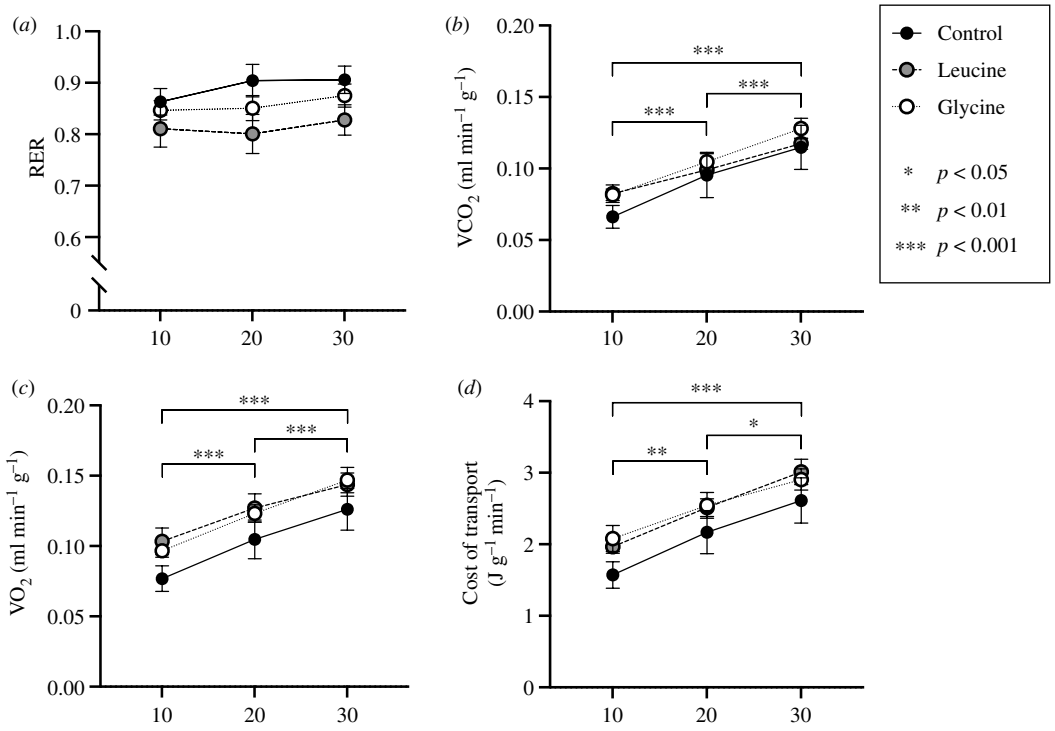

Figure 2. (*a*) The average respiratory exchange ratio (RER), (*b*) rate of carbon dioxide production (VCO_2), (*c*) rate of oxygen consumption (VO_2), and (*d*) cost of transport (CoT) in *Desmodus rotundus* at 10, 20 and 30 m min^{-1}. Asterisks denote significant differences between treadmill speeds when a significant main effect of 'speed' was detected (mixed effect model, $p < 0.05$). Error bars represent the standard error.

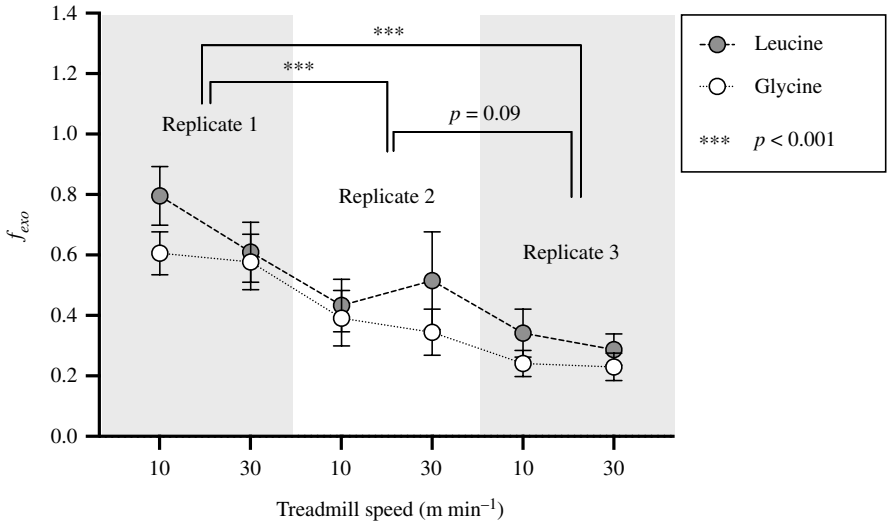

Figure 3. The proportion of exhaled CO_2 exhibiting the ^{13}C isotopic signature of the tracer-enriched blood meal (f_{exo}) at differing treadmill speeds and across the three exercise replicates. Reliance on the meal to fuel running was similar between amino acids and occurred very rapidly (during replicate 1) and then falling over time as the meal was assimilated (during replicates 2 and 3). Asterisks denote significant differences between exercise replicates because a significant main effect of 'replicate' was detected (mixed effect model, $p < 0.001$). Error bars represent the standard error.

Na^+, K^+ and H^+ ATPase activities than mammals of a similar size, facilitating rapid water absorption and acid secretion for protein hydrolysis [22]. Vampire bats possess limited capacities for regulating blood glucose levels during a fast and in storing energy in the form of glycogen or fats [25], suggesting their ability to sustain exercise using these energy stores is similarly limited. Instead, vampire bat skeletal muscle tissue seems capable of oxidizing amino acids from body stores and from ingested blood meals at remarkably high rates, based on the consistent RER values of 0.8–0.9, and the rapid and extensive reliance on oxidation of labelled ingested amino acids. Interestingly, flight is typically a far more energetically costly mode of locomotion in vertebrates than running [26], yet to our knowledge, no studies have explicitly investigated the contribution of a recently ingested blood meal to powering flight in vampire bats—an interesting avenue for future work.

More generally, our findings are consistent with other stable isotope experiments performed on bats with specialized, nectar-rich diets, that showed recently ingested sugars supplied >75% of the energy required to fuel hovering flight in the Pallas' long-tongued bat (*Glossophaga mutica*) just 30 min after feeding. By comparison, only 25–30% of a human's energy expenditure during exercise can be supported by a recently ingested sugars [27,28]. Much like vampire bats, nectivorous bats have specialized mechanisms to facilitate the rapid use of ingested sugar to fuel activity, such as high levels of intestinal sucrase for sucrose hydrolysis [29], paracellular pathways for intestinal sugar absorption [30] or high glucose transporter gene

expression [31]. Our findings highlight that bats possess highly efficient mechanisms for rapidly utilizing nutrients from their meals to meet the energy demands of their unique lifestyles.

Non-essential amino acids, including glycine, are well integrated into multiple cellular metabolic pathways. While glycine is a key substrate for purine production [32], it is also linked to pathways of ATP generation. Glycine can be produced from glucose via the conversion of glycolytic intermediate 3-phosphoglycerate to the amino acid serine and serine's conversion to glycine [33]. Alternatively, glycine can be catabolized to support ATP production via its interconversion with serine, and the further catabolism of serine to pyruvate, which can be transported into the mitochondria and further oxidized in the tricarboxylic acid (TCA) cycle [32]. While acknowledging this, the contribution of glycine/serine to ATP production in skeletal muscle during exercise is essentially undetectable in most mammals [34]. Instead, more glycine catabolized in mammals (e.g. approx. 40% of whole-body glycine flux in healthy humans [35] is processed through the glycine cleavage system, in which glycine is broken down, liberating CO_2 and ammonia [32], but not leading to substantial ATP synthesis [32]). Because the vast majority of whole animal CO_2 production during moderate to intense aerobic exercise comes from oxidation of metabolic substrates through the TCA, it seems improbable that the apparently high rate of glycine oxidation observed in running vampire bats is due to glycine cleavage system activity. Rather, our results suggest a dramatically enhanced capacity for the utilization of glycine, or related metabolic intermediates (e.g. serine) as aerobic fuels.

We found no evidence indicating that vampire bats discriminate between the amino acids oxidized during exercise. The f_{exo} values were similar between bats fed cow's blood enriched with isotopically labelled leucine and glycine. Most mammals possess the biochemical machinery to oxidize essential amino acids, particularly branch-chain amino acids such as leucine, to support exercise. Several experiments using isotopically labelled leucine infusions have shown increased rates of leucine oxidation in exercising mammals. Despite this, leucine oxidation typically accounts for less than 1% of total ATP turnover during exercise [36–39]. However, leucine is known to play other important roles in exercise metabolism. For example, leucine stored in the skeletal muscle can be catabolized to serve as a nitrogen donor for glutamine and alanine synthesis, both of which are readily oxidized during exercise to generate ATP [38]. In our study, the labelled meal accounted for as much as 60% of oxidized fuels in bats at peak usage. Our findings suggest that major enhancement of flux through these and related metabolic pathways has evolved in vampire bats as an adaptation to make efficient use of those fuels ingested in abundance (i.e. blood proteins and amino acids), marking a striking example of convergent evolution among both vertebrate and invertebrate obligate blood-feeding animals.

Ethics. Conducted under UofT Animal Care Protocol 20012113. Capture of bats conducted under Belize Forest Department Permit FD/WL/1/23 (1) with access to the Lamanai Archaeological Preserve under Belize Institute of Archaeology permit IA/H/23/01.

Data accessibility. All raw data are included as electronic supplementary material and available online [40].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. G.S.R.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; K.C.W.: conceptualization, funding acquisition, project administration, supervision, writing—review and editing.

Both authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. The funding for this work includes a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to K.C.W. (grant no. RGPIN-2020-06344), and an NSERC Postdoctoral Fellowship and L'Oreal-UNESCO Fellowship for Women in Science to G.S.R (grant no 557406-2021).

References

1. Brooks GA, Mercier J. 1994 Balance of carbohydrate and lipid utilization during exercise: the 'crossover' concept. *J. Appl. Physiol.* **76**, 2253–2261. (doi:10.1152/jappl.1994.76.6.2253)
2. Mul JD, Stanford KI, Hirshman MF, Goodyear LJ. Exercise and regulation of carbohydrate metabolism. In *Progress in molecular biology and translational science*, pp. 17–37, vol. **135**. Amsterdam, The Netherlands: Elsevier.
3. Carraro F, Naldini A, Weber JM, Wolfe RR. 1994 Alanine kinetics in humans during low-intensity exercise. *Med. Sci. Sports Exerc.* **26**, 348–353. (doi:10.1249/00005768-199403000-00011)
4. Rennie MJ, Edwards RHT, Krywawych S, Davies CTM, Halliday D, Waterlow JC, Millward DJ. 1981 Effect of exercise on protein turnover in man. *Clin. Sci*. **61**, 627–639. (doi:10.1042/cs0610627)
5. Teulier L, Weber JM, Crevier J, Darveau CA. 2016 Proline as a fuel for insect flight: enhancing carbohydrate oxidation in hymenopterans. *Proc. R. Soc. B* **283**, 20160333. (doi:10.1098/rspb.2016.0333)
6. Suarez RK, Darveau CA, Welch KC Jr, O'Brien DM, Roubik DW, Hochachka PW. 2005 Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *J. Exp. Biol*. **208**, 3573–3579. (doi:10.1242/jeb.01775)
7. Bursell E. 1963 Aspects of the metabolism of amino acids in the tsetse fly, *Glossina* (Diptera). *J. Insect Physiol*. **9**, 439–452. (doi:10.1016/0022-1910(63)90054-4)
8. Bursell E. 1975 Substrates of oxidative metabolism in dipteran flight muscle. *Comp. Biochem. Physiol. B Comp. Biochem.* **52**, 235–238. (doi:10.1016/0305-0491(75)90057-7)
9. Scaraffia PY, Wells MA. 2003 Proline can be utilized as an energy substrate during flight of *Aedes aegypti* females. *J. Insect Physiol*. **49**, 591–601. (doi:10.1016/s0022-1910(03)00031-3)
10. Voigt CC, Speakman JR. 2007 Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. *Funct. Ecol*. **21**, 913–921. (doi:10.1111/j.1365-2435.2007.01321.x)
11. Welch KC Jr, Herrera M LG, Suarez RK. 2008 Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga soricina*. *J. Exp. Biol.* **211**, 310–316. (doi:10.1242/jeb.012252)
12. Chen CCW, Welch KC. 2014 Hummingbirds can fuel expensive hovering flight completely with either exogenous glucose or fructose. *Funct. Ecol*. **28**, 589–600. (doi:10.1111/1365-2435.12202)

13. Welch KC, Myrka AM, Ali RS, Dick MF. 2018 The metabolic flexibility of hovering vertebrate nectarivores. *Physiology* **33**, 127–137. (doi:10.1152/physiol.00001.2018)
14. Riskin DK, Parsons S, Schutt WA Jr, Carter GG, Hermanson JW. 2006 Terrestrial locomotion of the New Zealand short-tailed bat *Mystacina tuberculata* and the common vampire bat *Desmodus rotundus*. *J. Exp. Biol.* **209**, 1725–1736. (doi:10.1242/jeb.02186)
15. Riskin DK, Hermanson JW. 2005 Independent evolution of running in vampire bats. *Nature* **434**, 292–292. (doi:10.1038/434292a)
16. Eberts ER, Dick MF, Welch KC. 2019 Metabolic fates of evening crop-stored sugar in ruby-throated hummingbirds (*Archilochus colubris*). *Diversity* **11**, 9. (doi:10.3390/d11010009)
17. Jeukendrup AE, Wallis GA. 2005 Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int. J. Sports Med.* **26**, S28–37. (doi:10.1055/s-2004-830512)
18. Bergman BC, Brooks GA. 1999 Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J. Appl. Physiol. (1985)* **86**, 479–487. (doi:10.1152/jappl.1999.86.2.479)
19. Hargreaves M, Spriet LL. 2020 Skeletal muscle energy metabolism during exercise. *Nat. Metab.* **2**, 817–828. (doi:10.1038/s42255-020-0251-4)
20. Zhou G *et al*. 2004 Metabolic fate of [^{14}C]-labeled meal protein amino acids in *Aedes aegypti* mosquitoes. *J. Insect Physiol.* **50**, 337–349.
21. Breidenstein CP. 1982 Digestion and assimilation of bovine blood by a vampire bat (*Desmodus rotundus*). *J. Mammal.* **63**, 482–484. (doi:10.2307/1380446)
22. Harlow HJ, Braun EJ. 1997 Gastric Na^+K^+ATPase activity and intestinal urea hydrolysis of the common vampire bat, *Desmodus rotundus*. *Comp. Biochem. Physiol. A Physiol.* **118**, 665–669. (doi:10.1016/s0300-9629(96)00464-1)
23. Schondube JE, Herrera-M LG, Martínez del Rio C. 2001 Diet and the evolution of digestion and renal function in phyllostomid bats. *Zoology* **104**, 59–73. (doi:10.1078/0944-2006-00007)
24. Blumer M *et al*. 2022 Gene losses in the common vampire bat illuminate molecular adaptations to blood feeding. *Sci. Adv.* **8**, eabm6494. (doi:10.1126/sciadv.abm6494)
25. Freitas MB, Welker AF, Millan SF, Pinheiro EC. 2003 Metabolic responses induced by fasting in the common vampire bat *Desmodus rotundus*. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **173**, 703–707. (doi:10.1007/s00360-003-0383-3)
26. Schmidt-Nielsen K. 1972 Locomotion: energy cost of swimming, flying, and running. *Science* **177**, 222–228. (doi:10.1126/science.177.4045.222)
27. Hawley JA, Dennis SC, Nowitz A, Brouns F, Noakes TD. 1992 Exogenous carbohydrate oxidation from maltose and glucose ingested during prolonged exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* **64**, 523–527. (doi:10.1007/BF00843762)
28. Jentjens RLPG, Shaw C, Birtles T, Waring RH, Harding LK, Jeukendrup AE. 2005 Oxidation of combined ingestion of glucose and sucrose during exercise. *Metab. Clin. Exp.* **54**, 610–618. (doi:10.1016/j.metabol.2004.12.004)
29. Hernandez A, Martinez del Rio C. 1992 Intestinal disaccharides in five species of phyllostomoid bats. *Comp. Biochem. Physiol. B Comp. Biochem.* **103**, 105–111. (doi:10.1016/0305-0491(92)90420-V)
30. Rodriguez-Peña N, Price ER, Caviedes-Vidal E, Flores-Ortiz CM, Karasov WH. 2016 Intestinal paracellular absorption is necessary to support the sugar oxidation cascade in nectarivorous bats. *J. Exp. Biol.* **219**, 779–782. (doi:10.1242/jeb.133462)
31. Camacho J *et al*. 2024 Sugar assimilation underlying dietary evolution of neotropical bats. *Nat. Ecol. Evol.* **8**, 1735–1750. (doi:10.1038/s41559-024-02485-7)
32. Kikuchi G. 1973 The glycine cleavage system: composition, reaction mechanism, and physiological significance. *Mol. Cell. Biochem.* **1**, 169–187. (doi:10.1007/bf01659328)
33. Amelio I, Cutruzzolá F, Antonov A, Agostini M, Melino G. 2014 Serine and glycine metabolism in cancer. *Trends Biochem. Sci.* **39**, 191–198. (doi:10.1016/j.tibs.2014.02.004)
34. Goodman MN, Ruderman NB. 1982 Influence of muscle use on amino acid metabolism. *Exerc. Sport Sci. Rev.* **10**, 1–26. (doi:10.1249/00003677-198201000-00001)
35. Lamers Y, Williamson J, Gilbert LR, Stacpoole PW, Gregory JF. 2007 Glycine turnover and decarboxylation rate quantified in healthy men and women using primed, constant infusions of [1,2-$^{13}C_2$]glycine and [$^2H_3$]leucine. *J. Nutr.* **137**, 2647–2652. (doi:10.1093/jn/137.12.2647)
36. White TP, Brooks GA. 1981 [U-14C]glucose, -alanine, and -leucine oxidation in rats at rest and two intensities of running. *Am. J. Physiol. Endocrinol. Metab.* **240**, E155–E165. (doi:10.1152/ajpendo.1981.240.2.E155)
37. Hagg SA, Morse EL, Adibi SA. 1982 Effect of exercise on rates of oxidation, turnover, and plasma clearance of leucine in human subjects. *Am. J. Physiol. Endocrinol. Metab.* **242**, E407–E410. (doi:10.1152/ajpendo.1982.242.6.E407)
38. Hood DA, Terjung RL. 1990 Amino acid metabolism during exercise and following endurance training. *Sports Med.* **9**, 23–35. (doi:10.2165/00007256-199009010-00003)
39. Lamont LS, McCullough AJ, Kalhan SC. 2001 Relationship between leucine oxidation and oxygen consumption during steady-state exercise. *Med. Sci. Sports Exerc.* **33**, 237–241. (doi:10.1097/00005768-200102000-00011)
40. Rossi GS, Welch KC. 2024 Supplementary material from: vampire bats rapidly fuel running with essential or non-essential amino acids from a blood meal. Figshare. (doi:10.6084/m9.figshare.c.7524683)