

## GAS EXCHANGE DURING HOVERING FLIGHT IN A NECTAR-FEEDING BAT *GLOSSOPHAGA SORICINA*

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### Summary

Glossophagine nectar-feeding bats exploit flowers while hovering in front of them. Aerodynamic theory predicts that power output for hovering flight in *Glossophaga soricina* is 2.6 times higher than during horizontal flight. We tested this prediction by measuring rates of gas exchange during hover-feeding. Five individuals of *Glossophaga soricina* (mean mass 11.7 g) were trained to feed from a nectar dispenser designed as a flow-through respirometry mask. Single hover-feeding events lasted for up to 4.5 s. Measured rates of gas exchange varied as a function of hovering duration.  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  during short hovering events (up to 1 s) were  $20.5 \pm 6.7 \text{ ml g}^{-1} \text{ h}^{-1}$  ( $N=55$ ) and  $21.6 \pm 5.6 \text{ ml g}^{-1} \text{ h}^{-1}$  ( $N=39$ ) (means  $\pm$  S.D.), respectively. These values are in the range of a previous estimate of the metabolic power input for level forward flight ( $23.8 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ). However, during

hovering events lasting longer than 3 s, oxygen uptake was only  $16.7 \pm 3.5 \text{ ml g}^{-1} \text{ h}^{-1}$  ( $N=73$ ), which is only 70 % of the value expected for forward flight. Thus, bats reduced their rate of oxygen uptake during longer periods of hovering compared with level forward flight. This result is in contrast to the predicted hovering cost derived from aerodynamic theory. The exact metabolic power input during hovering remains uncertain. During longer hovering events, bats were probably not in respiratory steady state, as indicated by the deviation of the respiratory exchange ratio from the expected value of 1 (oxidization of nectar sugar) to the measured value of 0.8.

Key words: gas exchange, respiration, hovering flight, *Glossophaga soricina*, bat, flight energetics, aerodynamics.

### Introduction

The neotropical Glossophaginae (Chiroptera, Phyllostomidae) are a group of bats which are highly specialized for consuming nectar and pollen from flowers. Convergent with nectar-feeders from other taxa, they have an elongated rostrum, reduced dentition and a long, protrusile tongue with a brush-tip for licking nectar from flowers (see Dobat, 1985; von Helversen, 1993). At night, bats spend 3–5 h foraging on the wing and exploit floral resources while hovering in front of the flowers (von Helversen and Reyer, 1984; von Helversen, 1986; Winter and von Helversen, 1998). Glossophagines have kinematic adaptations for hovering that are possibly unique among bats. During the wing's backstroke, the hand wing is sharply supinated so that its underside is turned upwards. Thus, even during backstroke, lift is produced by the distal tip of the wing (the 'distal wing triangle') (von Helversen and von Helversen, 1975; von Helversen, 1986). This adaptation may be understood as a functional evolutionary response to selection for economically efficient hover-feeding.

The energy required for hovering has not yet been quantified for *G. soricina*. It is necessary to determine this cost in order to understand the energetic requirements of generating the aerodynamic forces necessary to keep the animal aloft. In addition, the magnitude of this flight cost determines the

importance of energy expenditure during hovering in a cost/benefit analysis of foraging in nectar-feeding bats. The energetic cost of level forward flight was determined for *G. soricina* using an indirect method based on measurements of daily energy expenditure and 24 h flight-time budgets to be 1.63 W for an 11.7 g bat (Winter *et al.* 1993; Winter and von Helversen, 1998). This energy expenditure for flight constitutes approximately 50 % of a bat's total daily energy expenditure. As a first step to estimating hovering power requirements, Norberg *et al.* (1993) determined the wing morphology, hovering kinematics and induced velocity of the downward accelerated air of hovering *G. soricina*. They used aerodynamic theory to predict the mechanical aerodynamic power requirements (power output) for hovering and forward flight. According to that analysis, the power output for hovering should be 2.6 times greater than that for horizontal forward flight (Norberg *et al.* 1993).

In the present study, we used an empirical approach to estimate hovering flight costs by measuring rates of gas exchange during flight. Individuals of *G. soricina* were trained to feed from an artificial nectar-feeder converted to a respirometry mask. Respiratory gas exchange during hovering was determined from changes in  $O_2$  and  $CO_2$  concentrations in air drawn through the mask. To our knowledge, this is the first

direct measurement of gas exchange during hovering flight in a bat.

## Materials and methods

### Animals

The study was based on five individuals (two males, three females) of *Glossophaga soricina antillarum* (Rehn) (Phyllostomidae: Glossophaginae) (origin Jamaica) born and raised in our breeding colony of about 60 individuals maintained in a tropical greenhouse at Erlangen University. The bats in these facilities are well trained for flight as they fly about freely and feed by hovering. Mean body masses of the animals during the measurements were 12.8, 11.2 and 11.1 g (females) and 12.2 and 11.3 g (males), and the overall mean mass was 11.72 g.

### Flight cage

Measurements were conducted in a climate-controlled room within a flight chamber that measured 7 m × 0.7 m × 2 m (length × width × height). The walls and top cover of this chamber were made from polyethylene sheets. Temperatures during the measurements were approximately 22 ± 1 °C, relative humidity was 57% and air pressure was 0.975 kPa. The photoperiod was set to 12 h:12 h L:D. The body mass of a bat during the experiments could be monitored without handling the animal. The only roost available in the flight chamber was a piece of cork suspended from an electronic balance (Mettler PM 100, 0.001 g resolution). To rest, a bat always came to this balance roost from which the body mass data were transmitted to a computer. The body mass of a quiescent bat was stored as the mean of ten measurements with a precision of better than 0.01 g. The body mass during a specific hovering event was interpolated from the measurements taken before and after an activity bout.

### Nectar feeder and flow-through respirometry mask

The artificial feeder was an acrylic cylinder instrumented to function as a respirometry mask (Fig. 1). The nectar flow into this feeder was controlled by a computer that operated a stepping-motor syringe pump allowing the rate of nectar flow during a hovering visit to be controlled by the experimenter. Artificial nectar was an aqueous solution of glucose, fructose and sucrose with a concentration of 17% (w/w) (Winter and von Helversen, 1998). The opening of the feeder was well above (1.5 m) the solid floor to minimize the ground effect on hovering (Rayner and Thomas, 1991). The time and duration of a hovering event were recorded from the bat's interruption of an infrared light beam at the front edge of the feeder cylinder (Fig. 1). To prevent the bat from supporting some of its body weight by leaning on the feeder, the feeder opening was oriented downwards at an angle of 45°.

### Hover training

The natural duration of hovering by a glossophagine bat while at a flower or feeder is only between 0.3 and 1 s for a single hovering visit (Y. Winter and O. van Helversen, unpublished observations in the wild and in captivity; Tschapka, 1993; Maier, 1995) and it rarely exceeds a few

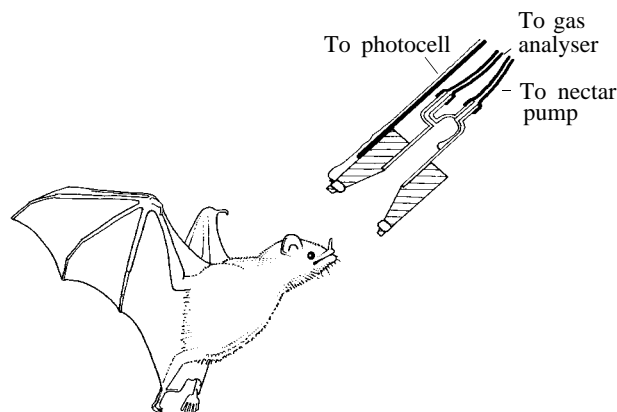


Fig. 1. The nectar-feeder functions as a flow-through respirometry mask. Air was drawn through the mask at a rate of 1000 ml min<sup>-1</sup> (STPD). The presence of a bat *Glossophaga soricina* at the feeder was detected by a photocell at the mask's opening. Artificial nectar was pumped into the feeder by a syringe pump driven by a stepping motor which, in turn, was controlled by a computer. The feeder opening was oriented downwards at 45° to prevent a hovering bat from supporting some of its body weight by leaning on the feeder (drawing by Holger Braun).

seconds. In fact, during our combined laboratory and field experience of working with *G. soricina*, we have never observed a hovering event even approaching 10 s in duration. For the experiments described here, we used a special food reward program to motivate the bats to hover for extended periods at the respirometry feeder. While the bat was hovering at the feeder, we provided the nectar food in the form of small successive portions of liquid instead of making the whole quantity available immediately upon the bat's arrival. Low rates of nectar flow led to an increase in hovering duration by the bat. During the training phase, we successively (i) increased the time interval between the bat's arrival at the feeder and the delivery of the first nectar portion, (ii) increased the time interval between delivery of subsequent portions of nectar and (iii) decreased the volume of a single food portion. During the respirometry measurements, the first nectar portion was delivered 500 ms after the bat's arrival at the feeder, and additional portions were given after each 500 ms. A single nectar portion had a volume of 5–12 µl.

### Respirometry

Air was drawn at a rate of 1000 ml min<sup>-1</sup> (STPD) through the mask. To set the flow rate at the feeder mask to a value at which all respiratory gases would be sucked into the system, the following experiment was performed. The flow rate at the feeder mask was reduced in seven steps between 1000 and 150 ml min<sup>-1</sup>. At each different flow rate, the oxygen uptake rates of a hovering *G. soricina* were measured. Data were then averaged to give one mean value of oxygen uptake rate ( $\dot{V}_{O_2}$ ) during hovering for each of the seven different flow rates. The plot of mean  $\dot{V}_{O_2}$  against flow rate at the feeder mask showed that  $\dot{V}_{O_2}$  measured during hovering remained constant at flow rates between 1000 and 300 ml min<sup>-1</sup>. Below 300 ml min<sup>-1</sup>, the measured rates of oxygen uptake decreased roughly in

proportion to flow rate. The flow rate used during the measurements reported here ( $1000 \text{ ml min}^{-1}$ ) was thus three times the minimum flow rate required to sample all respiratory gases. At the same time, this flow rate was low enough that the feeder mask did not act as a 'suction pump' on the bat's head. The free space remaining between the bat's head and the inner wall of the feeder mask was still several times the cross-sectional area of the 4 mm inner diameter gas tubing leading to the analyser. In addition, there was no indication of a temporarily reduced rate of gas flow during a bat's hovering visit at the mask (gas flow monitored using a mass flow meter).

The components of the respirometry system downstream from the mask were (in order): desiccant ( $3\text{\AA}$  molecular sieve), filter, mass flow controller (Bronkhorst F 111 C-HB), membrane pump (suction and pressure), bleeding valves, gas analyzers ( $\text{O}_2$  and  $\text{CO}_2$  in parallel), surge tanks (1 l), flow-measuring devices (Rotameter) and membrane pumps (suction). The gas analyzers used were Ametek (now AEI Technologies) S-3A/II with a dual N-37M sensor for oxygen differential measurement and a Hartmann & Braun URAS 10E for carbon dioxide. To adjust the  $\text{CO}_2$  concentrations to the range of sensitivity of the URAS 10E (0–500 p.p.m.), the sample air drawn from the mask was diluted with dry,  $\text{CO}_2$ -free air (NaOH as absorbent) at a ratio of 1:7 immediately before being drawn into the  $\text{CO}_2$  sensor. This gas mixing ratio was determined and kept constant using two mass flow controllers (Bronkhorst). The tubing was made from polyvinylchloride and polyurethane (Festo). The analogue signals from the gas analyzers were digitized (Sable Systems, 16-bit) and sampled at a rate of 4.5 Hz by a computer (MS-DOS/386).

The total respiratory gas volumes exchanged by a bat during single hovering visits were calculated by integrating the signal envelope of oxygen depletion or carbon dioxide enrichment over time. The rates of gas exchange were obtained by dividing the volume (in ml) of  $\text{O}_2$  or  $\text{CO}_2$  by the length of time during which the bat's head was inside the mask. Volumes of  $\text{O}_2$  were then corrected for the change in the air composition caused by the production of carbon dioxide by applying a correction equation derived for open-mask flow-through respirometry with  $\text{H}_2\text{O}$  absorbent but no  $\text{CO}_2$  absorbent (equation 3b in Withers, 1977). Measured gas volumes (ml per visit) were converted to rates ( $\text{ml g}^{-1} \text{ h}^{-1} \text{ STPD}$ ) by dividing by the hovering duration and the body mass of the animal. A special correction for STPD was not necessary as the air was dried before flow measurement, and mass flow controllers give flow rates in STP.

Before taking measurements, the complete system was calibrated by pulsed injections (using a stepping-motor syringe pump) of known volumes of  $\text{CO}_2$  and  $\text{N}_2$  ( $\text{N}_2$  dilution technique; Fedak *et al.* 1981) into the feeder mask. In addition, the calibration of the  $\text{CO}_2$  sensor was verified by injecting known concentrations of  $\text{CO}_2$  using a gas-mixing pump (Wösthoff, SA27/2). Using pulsed injections of small volumes of gas into the system (50, 100, 200 and  $400 \mu\text{l STPD}$ ), we simulated the measurement situation of a hovering bat which exchanged roughly  $50 \mu\text{l s}^{-1}$ . We found that both the  $\text{O}_2$  and the  $\text{CO}_2$  analysis systems showed a damped (due to system

wash-out), but otherwise completely linear, response to these transient peak changes in gas concentration. The volumes (integrals) of the signals corresponded (within  $\pm 3\%$ ) to the volumes of the injected gas pulses.

Because we wanted to know whether respirometry parameters differed between hovering events of different duration, we analyzed only single peaks that could be attributed to single hovering events rather than integrating over complete series of hovering bouts. Owing to the wash-out characteristics of the  $\text{CO}_2$  analysis system, however, the measured  $\text{CO}_2$  signals overlapped when the time interval between two successive hovering visits was less than 1 min. (Wash-out delay of the  $\text{O}_2$  analyzer was much shorter.) Using the following procedure, we were able to separate the signal traces of single visits from series of overlapping signals. This was made possible because the wash-out characteristics of our gas analysis system were constant during the experiments. Therefore, the declining slope of any  $\text{CO}_2$  pulse signal always followed the same course once it had dropped below its point of inflection (Fig. 2A). The course of this decline was virtually

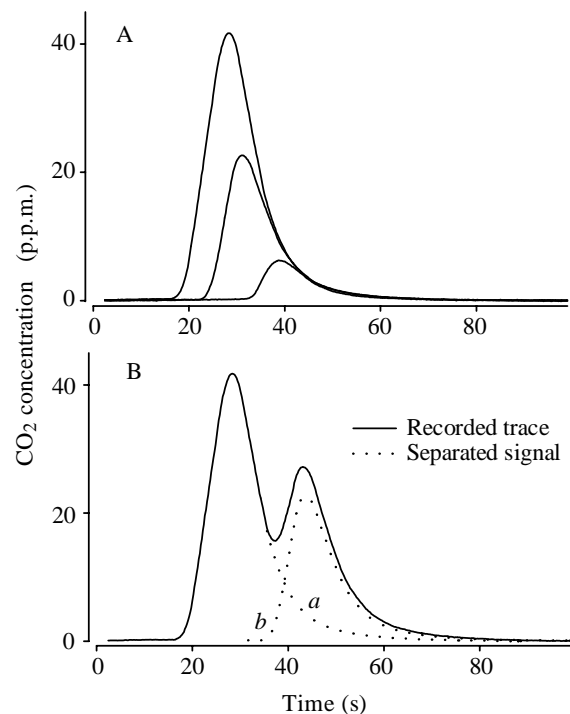


Fig. 2. The method used to separate individual  $\text{CO}_2$  signals from traces of overlapping signals. (A) Recordings of injections of three different volumes of  $\text{CO}_2$  into the gas analysis system. For this figure, the different signal traces were superimposed. Note that, once the declining slope of a signal has fallen below its inflection point, it follows a standard trajectory irrespective of the signal's maximum amplitude. This standard declining slope was used to complete individual  $\text{CO}_2$  traces when signals overlapped. (B) Individual  $\text{CO}_2$  signals were separated from traces of overlapping signals (solid line) by first completing the leading signal using the standard declining slope (dotted line *a*) and then subtracting this slope from the following main signal trace. Note that the maximum of the second signal is reduced (dotted line *b*) after the declining slope of the previous signal has been subtracted from the main trace.

independent of signal amplitude. We determined this standard declining slope for our gas analysis system from injections of CO<sub>2</sub> pulses with differing volumes delivered by hand into the analysis system (Fig. 2A). When signals from our experimental measurements overlapped, we completed the leading signal from its point of signal overlap using this standard signal decline and subtracted this same slope from the following signal in a stepwise procedure (Fig. 2B). When signals were too close and overlapped before the point of inflection (at peak-to-peak distances of less than 10 s), CO<sub>2</sub> signals could not be separated using this method. In this case, data points were obtained for  $\dot{V}_{O_2}$  without a corresponding  $\dot{V}_{CO_2}$  value so that a mean value for  $\dot{V}_{CO_2}$  (from Fig. 4B) had to be used for deriving  $\dot{V}_{O_2}$  from measured concentration differences (Withers, 1977). Results, unless stated otherwise, are given as the mean  $\pm 1$  standard deviation (S.D.).

#### Body mass support by the feeder mask

To test for the possibility that bats might support some of their weight on the feeder mask during hovering, we measured the vertical force exerted by the bat on the feeder during hovering. For this, the feeder mask was fixed onto the top of a programmable electronic Mettler PM-100 balance (with a metal wire 30 cm above the balance plate). The balance was set to its fast-response weighing mode, and the 90 % response time of the balance's force sensor was in the range 200–400 ms. Data were sent from the balance to a computer (via the serial port) at a rate of 7 Hz. To prevent the air accelerated downwards by the hovering bat from exerting a force on the balance plate, the plate was shielded from this air current by a piece of cardboard (with a small hole for the feeder holder). Blowing onto this cardboard shield from a distance of 20 cm increased the mass indicated by the balance by less than 10 mg. Measurements were performed for two individuals of *Glossophaga soricina* with a mean body mass during the measurements of 10.9 g. Hovering durations were timed from the bat's interruption of a vertical infrared light beam positioned approximately 5 cm in front of the feeder mask. These measurements were performed without simultaneous respirometry. Neither the photoelectric device nor the gas tubes were connected to the feeder mask to avoid the extra weight on the balance. For final data analysis, the force measurements during the last 250 ms of a hovering event were not included because the balance became unstable during the bat's departure.

### Results

Individual bats in the flight chamber were active throughout the night with a total flight duration of between 1 and 5 h per night. During this time, an individual made between 400 and 600 visits to the feeder and consumed 10–15 ml of nectar. During a visit to the feeder, the bat inserted its head up to the ears into the respirometry mask. As a consequence of the delayed nectar delivery, hovering duration increased from the typical value of less than 1 s to durations of up to 4.5 s. Bats

did not hover for longer durations even though they would have continued to receive nectar from the feeder.

Bats normally approached the feeder from below, with the flight path resembling the course of an upswinging pendulum with its 'dead centre' at the level of the feeder. Bats thus saved flight power by utilizing their kinetic energy during the final approach path.

Respirometry measurements were made during 357 hovering visits with a duration of between 0.2 and 4.4 s. The measured volumes of oxygen uptake (Fig. 3) and carbon dioxide output (results not shown) during a single hovering visit showed a linear relationship with hovering duration. Gas exchange per unit hovering time ( $\text{ml g}^{-1} \text{h}^{-1}$ ) varied with hovering duration (Fig. 4A). For a statistical comparison of short and long hover-feeding events, hover-feeding events were grouped into events shorter than 1 s and events longer than 3 s. Within these two groups, the five individuals did not differ in their mean values of oxygen uptake (one-way ANOVA,  $<1$  s,  $P>0.2$ ;  $>3$  s,  $P>0.1$ ), and data from all five individuals were therefore combined. Mean  $\dot{V}_{O_2}$  ( $\pm 1$  S.D.) during short hovering visits of up to 1 s was  $20.5 \pm 6.7 \text{ ml g}^{-1} \text{h}^{-1}$  ( $N=55$ ) or  $240 \text{ ml h}^{-1}$  for the 11.7 g bats. During longer hovering events lasting between 3 and 4.4 s, this rate decreased to a mean value of  $16.7 \pm 3.46 \text{ ml g}^{-1} \text{h}^{-1}$  ( $N=73$ ) or  $195 \text{ ml h}^{-1}$ , which was significantly lower than that during short hovering visits (Mann–Whitney rank sum test,  $P<0.001$ ). Bats thus reduced their rate of gas exchange during longer hovering visits.  $\dot{V}_{CO_2}$  was similar to  $\dot{V}_{O_2}$  during the short hovering events of up to 1 s duration ( $21.6 \pm 5.57 \text{ ml g}^{-1} \text{h}^{-1}$ ,  $N=39$ , or  $253 \text{ ml h}^{-1}$ ). The respiratory exchange ratio ( $\text{RER} = \dot{V}_{CO_2} / \dot{V}_{O_2}$ , determined from the expired air, as opposed to the cellular respiratory quotient RQ) was therefore approximately 1 ( $1.16 \pm 0.27$ ,  $N=39$ , Fig. 4B). During longer hovering visits,  $\dot{V}_{CO_2}$  decreased even further than  $\dot{V}_{O_2}$ . For hovering durations between 3 and 4.4 s mean,  $\dot{V}_{CO_2}$  was  $13.1 \pm 2.28 \text{ ml g}^{-1} \text{h}^{-1}$

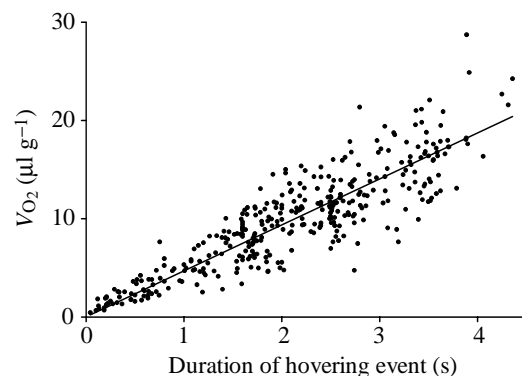


Fig. 3. Oxygen uptake (STPD) ( $\dot{V}_{O_2}$ ) of five bats *Glossophaga soricina* ( $N=357$  foraging visits, mean mass 11.7 g) during hovering flight at a nectar-feeder functioning as a respiratory mask. Data are total oxygen uptake during single hovering events. Hovering duration was timed by the computer using an infrared sensor at the front edge of the mask. The solid line is the regression ( $\dot{V}_{O_2} = 4.68t$ , where  $\dot{V}_{O_2}$  is in  $\mu\text{l g}^{-1}$  and  $t$  is time in s,  $N=357$ ,  $r^2=0.78$ ,  $P<0.001$ ). Data can be converted to whole-animal units by multiplying by the mean body mass of 11.7 g.



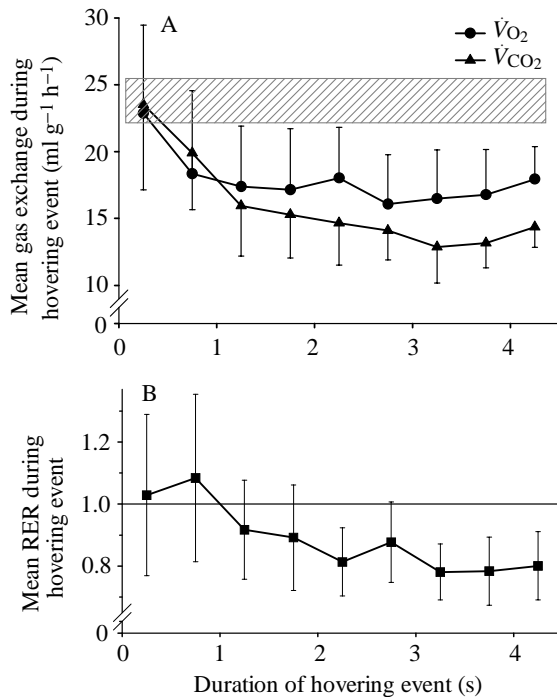


Fig. 4. Mean gas exchange rates during hovering events of differing durations. Values are means  $\pm$  s.d.,  $N=5$ . (A) Oxygen uptake ( $\dot{V}O_2$ ) and carbon dioxide output ( $\dot{V}CO_2$ ); (B) respiratory exchange ratio (RER) ( $\dot{V}CO_2/\dot{V}O_2$ ) of *Glossophaga soricina* (mean mass 11.7 g) during hovering flight at a respiratory mask. RER was significantly different from the expected steady-state value of 1 (horizontal line in B) only during hovering flights longer than 2 s (paired-sample  $t$ -test against variable with value 1,  $P<0.001$ ). Data are the mean values over whole hovering events of differing duration. Rates were computed from the measured volumes of gas exchange (in STPD) during single hovering events given in Fig. 3 by dividing by hovering duration. The shaded area in A indicates, for comparison, their rate of oxygen uptake ( $23.8 \pm 1.8$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) during level forward flight at a medium flight speed as determined previously for 11.7 g *G. soricina* by an indirect method (Winter *et al.* 1993; Winter and von Helversen, 1998). Data can be converted to whole-animal units by multiplying by the mean body mass of 11.7 g.

( $N=53$ ) or 153 ml h<sup>-1</sup>, and the RER fell to  $0.79 \pm 0.1$  ( $N=53$ , Fig. 4B). It should be noted that these values for  $\dot{V}CO_2$  and RER (as shown in Fig. 4) are the mean values for whole hover-feeding events. If the instantaneous RER during a 4 s hovering event was also approximately 1 at the onset of hovering, then it must have dropped during the hovering event to significantly below the overall mean value of 0.8.

Measurements of the vertical force exerted by a bat onto the feeder mask determined using an electronic balance yielded the following result. At the beginning of a hovering event, when a bat pushed its head into the feeder mask from below, it produced an upwardly oriented force (lift) against the mask that ranged (for the two individuals) between  $-6$  and  $-22$  mN (Fig. 5). Within the first second of hovering, this lift force declined to a value of approximately zero and then turned into a downwardly oriented force (indicating that the bat was

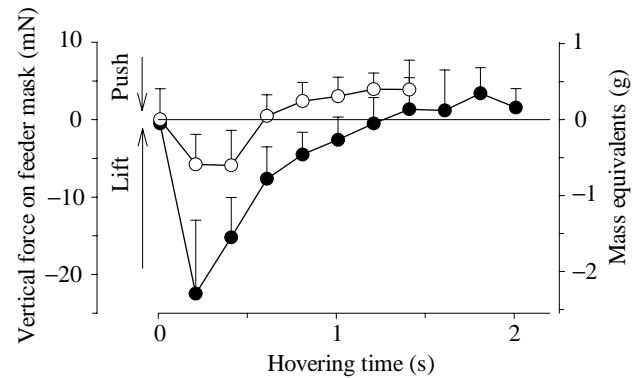


Fig. 5. The vertical force exerted on the feeder mask by two hovering *Glossophaga soricina*. Negative values indicate the exertion of an upward-oriented force (lift) onto the mask. Bats produced an upward lift force while inserting their head into the mask (force significantly less than 0). Data are means  $\pm$  1 s.d. from two individuals (open circles,  $N=84$ , hovering duration 1.3–1.9 s, mass 11.3 g; filled circles,  $N=22$ , hovering duration 1.7–2.3 s, mass 10.5 g). Forces were significantly greater than 0 ( $P<0.001$ ) after 1 s (open circle) or 1.5 s (filled circle) of hovering flight (paired-sample  $t$ -test against variable with value 0). Force measurements during hovering (sampled at a rate of 7 Hz) were performed with the feeder mask (without the respirometry tube or photocell electronic wiring) connected to the weighing plate of an electronic balance (Mettler PM-100). The opening of the feeder mask was oriented downwards at 45°, and the mask was positioned approximately 30 cm above the balance. The balance was shielded from the air accelerated downwards by the hovering bat. Data from the last 0.25 s of a hovering event were not included as the balance became unsteady during the bat's take-off.

leaning on the mask). The magnitude of the downward-oriented force after 1–2 s of hovering, however, did not exceed 3.9 mN, which is equivalent to a body mass support of less than 0.4 g. Thus, even after the initial period of hovering, bats supported less than 4% of their body mass by leaning on the feeder mask (when this was oriented downwards at 45°).

## Discussion

The energy expenditure during forward flight at medium speed in *G. soricina* has been estimated at  $1.63 \pm 0.12$  W (Winter *et al.* 1993; Winter and von Helversen, 1998) for individuals of the same mean body mass (11.7 g) as used during the respirometry measurements described here. The estimate of forward flight cost was based on an indirect method in which both daily energy expenditure and the total amount of flight activity during 24 h were quantified. Flight cost was then estimated by relating 24 h flight activity with daily energy expenditure using multiple regression analysis and taking into account resting metabolic expenditure. The forward flight cost obtained in that study is within the range of estimates of flight cost in small vespertilionid bats obtained using a different method (doubly labelled water method, Speakman and Racey, 1991) and it coincides with estimates that have since been determined for five other species of glossophagine bats ranging

in body mass from 7 to 28 g (Winter and von Helversen, 1998). At a metabolic RQ of 1 (using sugar as the substrate for catabolism during the active phase), the power input of  $1.63 \pm 0.12$  W during forward flight corresponds to  $23.8 \pm 1.75$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> for the 11.7 g bats. In the present study, mean  $\dot{V}_{O_2}$  during short hovering visits of up to 1 s duration ( $20.5 \pm 6.7$  ml g<sup>-1</sup> h<sup>-1</sup>, see Fig. 4A) was only 15 % below  $23.8$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> and was thus within the range of values for forward flight power. This agreement between the oxygen uptake rates during forward flight and short hovering events is expected because, directly after a switch from one work level to another, the respiratory rate should initially reflect the rate of the previous work level. The RER of  $1.16 \pm 0.27$  determined from respiratory gases during short hovering events (Fig. 4B) also does not differ significantly from the expected cellular RQ of 1 for steady-state respiration. Measured  $\dot{V}_{O_2}$  during short hovering visits was thus in general agreement with the rate expected during forward flight. In contrast, the low rate of oxygen uptake during longer hover-feeding events, as measured here, was an unexpected finding.

Norberg *et al.* (1993) predicted that aerodynamic power output for hovering would exceed the corresponding value for horizontal forward flight by a factor of 2.6. This coincides with conventional aerodynamic theory which predicts a U-shaped relationship between flight power and flight speed so that power output for flight should rise at low flight speeds below the minimum power speed (e.g. Weis-Fogh, 1972, 1973; Pennycuik, 1975, 1989; Norberg, 1990; Rayner, 1979a,b,c; Ellington, 1984). This is because, at zero forward speed, the surface area swept by the wing in a unit of time to accelerate air downwards is much smaller than for a moving animal during forward flight. This smaller air volume must therefore be accelerated to a higher velocity to generate the required weight support. Since power equals the air mass times the square of the downward velocity, this requires more power (Norberg, 1990; Norberg *et al.* 1993). If, as an approximation, we assume the same metabolic efficiency for forward and hovering flight, then the oxygen demand for hovering flight, as predicted by the aerodynamic analysis, should equal 2.6 times  $23.8$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>, which is  $62$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>. Contrary to our expectations, the measurements obtained here showed that, during longer hovering visits (>3 s), *G. soricina* reduced its rate of oxygen uptake below the value expected for forward flight and also below the value measured during shorter (<1 s) hovering events.  $\dot{V}_{O_2}$  during hovering events of longer than 3 s ( $16.7$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) was only 27 % of the oxygen demand extrapolated from aerodynamic theory. Although we cannot presently resolve this discrepancy, we can discuss a number of aspects that may be of relevance. Two questions need to be addressed. (1) Is the metabolic power required for hovering in reality higher than that reflected by the results of the measurements of gas exchange? (2) Or is hovering power near the range of values measured here and thus much lower than predicted by aerodynamic considerations?

There are two potentially energy-saving mechanisms that could be exploited by the bats to reduce their power

requirements during hovering. First, by approaching the feeder like an upswinging pendulum, bats save hovering flight power by utilizing kinetic energy during the final approach to the feeder. While at the 'dead centre' of the swing, at least for a short time, they remain in the air at minimum expense. The duration of this near stand-still, however, should be very short and, probably, not have an effect beyond a few tenths of a second. We therefore consider it unlikely that this mechanism could lead to major energy savings during extended hovering. In fact, the upward-oriented vertical force exerted by the bat during the beginning of a hovering event at the feeder mask (Fig. 5) indicates that an arriving bat used its 'swing' (its kinetic energy) to push its head into the feeder mask. Second, bats supported some of their body mass by leaning on the respirometry mask after the initial period of hovering (Fig. 5). However, the downward-exerted force of less than 4 mN corresponded to a mass support of less than 0.4 g, which was less than 4 % of the mean body mass. The magnitude of this effect was therefore fairly small and cannot explain the low values of oxygen uptake measured here.

The gas exchange transients between the lung and the atmosphere reflect events taking place in the tissues in a distorted manner because of the intervening circulatory delays and the buffering effects of gas stores. We had hoped to derive an estimate of the final steady-state condition of the gas exchange system from the dynamic behaviour of the *change* in the mean gas exchange rate during single hovering flights of differing duration. Such changes, however, were not observed. Even during hovering flights of more than 4 s duration (equal to approximately 60 breathing cycles), the mean  $\dot{V}_{O_2}$  during a hovering event did not show a significant upward trend compared with shorter hovering events. This is surprising if the metabolic power released during hovering really required an oxygen uptake four times higher than measured here.

Metabolic power can only be inferred from respiratory  $\dot{V}_{O_2}$  when respiration is in equilibrium with metabolic processes. In this situation, the respiratory exchange ratio is also in equilibrium with metabolic RQ at the cellular level. During the night, nectar-feeding bats live on a sugar diet, and metabolic RQ should therefore equal 1, as is the case in other nectar-feeders such as hummingbirds (Suarez *et al.* 1990). An RER of approximately 1 was measured during short hovering visits, but it decreased to 0.8 for hovering events lasting longer than 3 s duration. This deviation of the RER from 1 is an indication that respiration during longer hovering events was possibly not in steady state with metabolic processes.

Bats have exceptionally large skin areas and may lose carbon dioxide through their well-vascularised, thin flight membranes. During hovering flight, cutaneous gas exchange should, however, amount to less than 1 % of the total carbon dioxide output and thus cannot explain the observed deviation in RER. This estimate of less than 1 % results when the maximum cutaneous CO<sub>2</sub> output rate that has been measured in a bat at a sublethal temperature at which the blood vessels of the flight membrane are engorged ( $56$  ml CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>, Herreid *et al.* 1968) are related to the wing area ( $0.0107$  m<sup>2</sup>,

Norberg *et al.* 1993) and  $\text{CO}_2$  output during hovering ( $153 \text{ ml h}^{-1}$ ; this study) of *G. soricina*.

One transient effect is the build-up of an oxygen debt after a stepwise increase in work load. After a sudden increase in power production, ATP resynthesis will deplete the oxygen stores in the tissue. This oxygen consumption is not immediately reflected at the lung level and would therefore not be measured as a change in RER over the same time scale. Another transient mechanism that could explain the change in RER is hypoventilation. Hypoventilation leads to a transient reduction of  $\text{CO}_2$  exchange because the  $\text{CO}_2$ -binding curve of the blood is much steeper than the corresponding  $\text{O}_2$ -binding curve. Thus, a change in pulmonary partial pressures, even if approximately equal in absolute values, such as that caused by hypoventilation, will initially have only a small effect on the alveolar  $\text{O}_2$  exchange rate, whereas  $\text{CO}_2$  exchange will be transiently suppressed (e.g. Scheid, 1996; Widdicombe and Davies, 1991). Hypoventilation could thus be a mechanism that might explain, for the short-duration phenomena investigated here, the observed pattern of a reduced  $\text{O}_2$  uptake rate with a concomitant drop in RER. It should be added as a cautionary note, however, that although these mechanisms may explain our observation, the RER is influenced by many factors so that it is generally not possible strictly to deduce from changes in RER any specific mechanisms involved.

The possibility that nectar-feeding bats may hypoventilate during hover-feeding is astonishing. It is not at all obvious why feeding should affect respiration mechanics. While it is a familiar phenomenon for adult humans that we cannot simultaneously breathe and swallow, this is the exception rather than the rule among mammals (Tillmann and Wustrow, 1982). (The human peculiarity is a necessary prerequisite for the development of a highly specialized vocal organ). Possibly, bats could down-regulate respiration for reasons other than drinking nectar. One reason could be that glossophagines have evolved a general tendency to reduce their breathing rate while keeping their heads within flowers. This might be, for example, to avoid the inhalation of fine pollen grains. Another reason could stem from the fact that the small, bell-shaped flowers, typical of some glossophagine-pollinated plants, are often no larger than a 'head-mask' for the bat (Vogel, 1968, 1969; von Helversen, 1993, 1995): the bat's head may virtually seal the corolla opening during hover-feeding. Given the resulting highly impeded flow of fresh air into the flower corolla, the limited gas exchange possible might not be worth the ventilatory effort.

Rates of oxygen uptake during hovering have been determined in hummingbirds and hawkmoths. Mean  $\dot{V}_{\text{O}_2}$  in hovering hummingbirds, including a 10 g *Eulampis jugularis*, ranges between 40 and  $45 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (for references, see Epting, 1980; Bartholomew and Lighton, 1986). For hawkmoths, Bartholomew and Casey (1978) determined the allometric scaling of hovering power on body mass (based on measurements from insects with body masses up to 3.4 g). Extrapolation to a hypothetical 11.7 g hawkmoth yields a hovering  $\dot{V}_{\text{O}_2}$  of  $37 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  ( $435 \text{ ml O}_2 \text{ h}^{-1}$  or  $2.59 \text{ W}$ ). Rates of hovering oxygen uptake in the two groups are thus

significantly higher than the  $\dot{V}_{\text{O}_2}$  of  $17\text{--}22 \text{ ml g}^{-1} \text{ h}^{-1}$  measured for hovering *G. soricina* in the present study. Hummingbirds and large hawkmoths differ from glossophagine nectar-feeding bats in that they can sustain hovering flight for extended periods of up to several minutes. Power input in these two groups can therefore be directly inferred from measurements of steady-state rates of oxygen consumption. In hovering *G. soricina*, however, respiration during the relatively short hovering flights was possibly not at steady state. For this reason, we urge caution in interpreting the findings of the present study as representing a precise estimate of metabolic power input during hovering. While this study represents a necessary first step in determining metabolic power input during hovering in *G. soricina*, additional measurements may be needed to unravel the picture completely.

In conclusion, the discrepancy between the low rates of measured oxygen uptake during hovering flight in *G. soricina* and the high values predicted from aerodynamic considerations cannot be resolved at this time. While it is possible that, during hover-feeding, the bats went into some type of oxygen debt (for example due to hypoventilation), it is also possible that the power required for hovering flight is lower than expected or that both of these factors play a role.

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