High activity enables life on a high-sugar diet: blood glucose regulation in nectar-feeding bats

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High blood glucose levels caused by excessive sugar consumption are detrimental to mammalian health and life expectancy. Despite consuming vast quantities of sugar-rich floral nectar, nectar-feeding bats are long-lived, provoking the question of how they regulate blood glucose. We investigated blood glucose levels in nectar-feeding bats (Glossophaga soricina) in experiments in which we varied the amount of dietary sugar or flight time. Blood glucose levels increased with the quantity of glucose ingested and exceeded 25 mmol l⁻¹ blood in resting bats, which is among the highest values ever recorded in mammals fed sugar quantities similar to their natural diet. During normal feeding, blood glucose values decreased with increasing flight time, but only fell to expected values when bats spent 75 per cent of their time airborne. Either nectar-feeding bats have evolved mechanisms to avoid negative health effects of hyperglycaemia, or high activity is key to balancing blood glucose levels during foraging. We suggest that the coevolutionary specialization of bats towards a nectar diet was supported by the high activity and elevated metabolic rates of these bats. High activity may have conferred benefits to the bats in terms of behavioural interactions and foraging success, and is simultaneously likely to have increased their efficiency as plant pollinators.

Keywords: blood glucose; glycated haemoglobin; HbA1c; hyperglycaemia; nectarivory; plant pollinator coevolution

1. INTRODUCTION

A large number of studies have shown that elevated blood glucose levels and high post-feeding blood glucose spikes caused by excessive sugar consumption are hallmarks of diabetes mellitus. They are also linked to decreased insulin sensitivity and multiple subsequent disorders, including nephro-, neuro- and retinopathy, and ultimately to reduced life expectancy in mammals [1–4]. This process, with its wide array of detrimental effects on health, is called glucotoxicity, and it has been well studied in humans and various animal models [5,6].

Nectar specialists among mammals benefit from having an energy-rich food source, but at the same time ingest unusually large quantities of glucose, and it is unclear how they escape the pathological side-effects of their diet [7]. Neotropical glossophagine bats are specialized nectar feeders that exploit floral nectars of many coevolved plants, providing pollinator services at the same time [8]. Floral nectar consists of a solution of glucose, fructose and sucrose in concentrations of up to 30 per cent [9]. Nectar-feeding bats consume large quantities of sugar each night, amounting to about one-quarter of their body mass [10–12]. Despite such high sugar consumption, they are relatively long-lived, given their small size: a 7–23 g bat may live for more than 10 years, which is at least five times longer than a rodent of similar size [13,14].

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In addition to their unusual diet, nectar-feeding bats have higher mass-specific metabolic rates than other similar-sized mammals with different feeding habits [10,11,15], arising from their costly foraging mode of exploiting flowers on the wing [16]. They have been found to rapidly absorb ingested nectar, being well equipped with intestinal sucrase, an enzyme responsible for the hydrolysis of sucrose [17]. Extraordinarily, the ingested sugars are then directly used, rather than being converted into fat, and almost entirely fuel the animals’ metabolism both at rest and during flight. This adaptation has probably evolved to spare body fat, and thus reduce body mass and associated additional flight costs, while also leading to high dietary efficiency, as the energetic costs of metabolic sugar–fat conversion are avoided [18,19]. The metabolic pathway of the sugar ingested in floral nectar between the time of ingestion and mitochondrial energy conversion is, however, unclear.

In nectar-feeding bats, the energetic costs of flight exceed those of rest by a factor of 10–12 [7,16]. To directly fuel these high energetic costs almost exclusively with exogenous glucose would require a constant and sufficiently high supply of glucose via the bloodstream, a prerequisite apparently easily achieved considering the high rate of sugar consumption in nectarivorous bats. However, the bats feed intermittently over the course of up to 12 h in nature, during which time they frequently alternate between energetically costly flight and rest [20]. In mammals, blood glucose levels are usually tightly

Accepted 22 March 2011
Published online 13 April 2011

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regulated, including during food uptake or physical exercise, and rarely exceed 10 mmol glucose l\(^{-1}\) blood [21–23]. To prevent high levels of circulating plasma glucose while simultaneously supplying their metabolism with sufficient exogenous glucose during activity and rest, nectarivorous bats require a highly effective regulatory feedback mechanism. Such a mechanism could potentially be primarly via either muscular or hepatic glycogenolysis and glycogenesis. Here, we asked how nectar-feeding bats manage to escape the adverse side-effects of extreme sugar consumption and associated postprandial elevated blood glucose levels, considering their extraordinary diet and energetic requirements. In a series of experiments, we investigated blood glucose regulation in the 10 g nectar-feeding bat *Glossophaga soricina*. We evaluated its rate of post-feeding blood glucose regulation during rest and flight activity, and its dependence on the amount of sugar in the diet.

2. METHODS

We conducted glucose tolerance tests on nectar-feeding bats, *G. soricina* (Phyllostomidae: Glossophagidae), from a captive breeding colony at the University of Erlangen, Germany. In nature, this common species occurs in a wide variety of habitats, from northern Mexico to northern Argentina, and feeds on the nectar of a large number of bat-pollinated plants [24]. Between experiments, the animals were maintained on honey water, hummingbird food (Nektar PLUS, Günther Enderle Nekton, Pforzheim, Germany) and baby food (Aleteamil, Alete, Nestlé, Germany) as a 20 per cent solution in tap water. Additionally, pollen and bananas were provided and water was available *ad libitum*. All animals fasted for at least 10 h prior to the experiments. We measured the bats' body mass (\(M_b\)) before each experiment (PM-300, Mettler, Greifensee, Switzerland) and mean \(M_b\) of the animals was 9.8 ± 0.7 g (\(n = 79\)).

To assess blood glucose levels during inactivity after feeding, we provided each bat with a single oral dose of glucose as a 21 per cent glucose solution (w/w), which the bats ingested spontaneously from a syringe, while being held in the hand. We tested animals with 1.8 (\(n = 5\)), 5.4 (\(n = 8\)) and 9 (\(n = 5\)) g glucose kg\(^{-1}\) \(M_b\). These amounts of glucose were relatively conservative estimates of what bats ingest in their natural habitat and were similar in volume and energy to the nectar from one to six flowers, or equivalent to 1 to 3 per cent of daily energy expenditure [21–23]. Blood glucose levels were always measured directly before feeding from the hand. (iii) To simulate most closely the conditions free-ranging animals would experience in their natural habitat, we fed each bat 5.4 g sugar kg\(^{-1}\) \(M_b\) in a 20 per cent solution at 15 min intervals for a total of 120 min. The sugar solution consisted of a mixture of glucose, fructose and sucrose (37 : 37 : 26%) in water, which approximated the quantity, concentration and sugar content of the nectar reward of two to four flowers, a conservative estimate for feeding rates in nature [7,9]. We measured the blood glucose of each bat before feeding at 15 min intervals. After feeding, the bats were allowed to fly for either 30, 45, 60 or 75 per cent of the subsequent 15 min time interval (\(n = 6\) bats per group). Flight times were divided into two periods of equal duration. When not in flight, the bats rested in cotton bags. All experiments were conducted during the first hours of the bats’ normal nocturnal activity period. Ambient temperature and humidity were stable throughout (approx. 25 °C, 50–60% relative humidity). We marked all bats by fur clipping after an experiment to ensure no animal was used twice. All sample sizes refer to numbers of individual bats tested.

Blood glucose may irreversibly react with haemoglobin, forming glycated haemoglobin (HbA1c). The extent of haemoglobin glycation strongly correlates with the level of ambient glycaemia during a period of several weeks, depending on the lifespan of erythrocytes and the degradation rate of HbA1c. Therefore, the percentage of haemoglobin that is glycated is a measure of mean blood glucose concentration over a longer time period [25,26]. We photometrically measured the percentage of HbA1c in the blood of *G. soricina* during the resting period with a Siemens DCA 2000+ (Siemens, Eschborn, Germany).

3. RESULTS

At the beginning of the experiments, blood glucose concentrations of fasting and resting bats were 3.0 ± 1.5 mmol l\(^{-1}\) blood (\(n = 79\)). After feeding, blood glucose concentrations increased rapidly and peaked 10–30 min post-feeding (figure 1). The magnitude of blood glucose spikes, as well as the time blood glucose levels remained elevated, correlated strongly with the quantity of glucose ingested (blood glucose peaks: Spearman rank \(r_s = 0.94\),
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p < 0.001; blood glucose integral: Spearman rank $r_s = 0.96$, $p < 0.001$; $n = 25$). After ingesting a single bolus of 9 g glucose kg$^{-1}$ $M_b$, maximum blood glucose concentrations exceeded 25 mmol glucose l$^{-1}$ blood after 30 min and mean values remained at around 15 mmol glucose l$^{-1}$ blood 90 min after glucose ingestion.

When we allowed the bats to fly for different time intervals after ingesting a single dose of 5.4 g glucose kg$^{-1}$ $M_b$, similar to the ingestion of nectar from two to four flowers, blood glucose peaks were lower and time integrals of blood glucose were smaller with increasing flight time (Pearson’s correlation, peaks: $r = -0.487, n = 32$, $p < 0.005$; integrals: $r = -0.825, n = 32$, $p < 0.001$; figure 2). Thus, flight lowered spikes of post-feeding blood glucose levels by increasing the clearance of glucose from the blood compared with resting bats. At 30 min after feeding, blood glucose was significantly lower in bats flying for 50 and 70 per cent of the time than in resting conspecifics (one-way ANOVA: $F_{3,34} = 26.73, p < 0.001$; post hoc Tukey’s test $Q = 8.56$ and 7.48, both $p < 0.001$). Therefore both the amount of glucose ingested and the time spent airborne determined blood glucose levels.

The ingestion of glucose with an energy content of about 1 per cent of daily energy expenditure (the equivalent of the nectar of approx. 2.5 flowers) every 10 min, however, resulted in increasing blood glucose levels, which reached on average 30 mmol l$^{-1}$ blood after 80 min, despite an intermittent flight activity of 20 per cent of the time (figure 3).

When we fed the bats a sugar solution simulating floral nectar rewards in sugar composition, concentration and volume of two to four flowers every 15 min, and subjected bats to different flight times between feeding, blood glucose levels tended to either level off or gradually decline after 15–60 min post-feeding (figure 4). The longer the bats remained airborne between feeding events, the faster the blood glucose values stabilized. Mean blood glucose levels over the last 60 min of the experiment were negatively related to flight time (Spearman rank $r_s = -0.73$, $p < 0.001$, $n = 24$), but only consistently reached values below 10 mmol glucose l$^{-1}$ blood when the bats spent 75 per cent of their time on the wing.

The percentage of HbA1c in the blood of $G. soricina$ was $3.9 \pm 0.3$ per cent ($n = 9$).

4. DISCUSSION

We found the nectar-feeding bat $G. soricina$ to express highly variable blood glucose levels and extreme peak and plateau values after glucose uptake in amounts similar to the animals’ normal diet. Our results show that bats resting directly after feeding experience hyperglycaemic spikes the magnitude of which increased with the amount of glucose ingested. Only through sustained, energetically costly flight for about 60 to 75 per cent of their time were the bats able to regulate blood glucose at values commonly found in mammals of that size [21–23]. This implies that $G. soricina$ does not (and is possibly unable) to regulate its post-feeding blood glucose levels exclusively by insulin-triggered cellular glucose transport as tightly as other mammals.

These observations are exceptional, as, in mammals, the precise regulation of blood glucose levels is considered crucial because of the highly detrimental effects of elevated blood glucose on health [1–4,22]. Therefore, blood glucose levels are usually regulated within tight margins, and are independent of dietary uptake or the degree of energy expenditure through physical activity [21–23]. Generally, blood glucose levels are negatively correlated with a species’ body mass, but values far above 10 mmol glucose l$^{-1}$ blood are unusual in small mammal species [21,23,27]. Indeed, blood glucose levels of over 25 mmol glucose l$^{-1}$ blood in $G. soricina$ were among the highest ever recorded in mammals fed quantities of glucose corresponding to their natural dietary uptake [10,11]. Such levels would be considered pathological in humans and other mammals [21,23,28].

Two scenarios may explain our findings: one is that high blood glucose levels, despite being above the
mammalian norm, are not harmful for nectar-feeding bats. An alternative but not mutually exclusive scenario is that, in nature, high flight activity largely prevents increased blood glucose levels after feeding.

It is conceivable that nectar-feeding bats, exceptionally among mammals, have adapted to tolerate high blood glucose levels. This, however, would require the bats to have evolved mechanisms, as yet unknown, to cope with the various consequences of hyperglycaemia that are usually noxious to mammalian health. Firstly, high blood glucose increases the non-enzymatic glycosylation of proteins and the formation of toxic advanced glycogen endproducts (AGE) such as HbA1c [25,26], which may cause various health defects (e.g. cardiovascular disease and diabetes [1–6]). Our finding of percentages of HbA1c within the expected range for mammals [29,30], however, implies that mean blood glucose levels were low over extended time periods, unless the rates of formation or deterioration of HbA1c differ markedly from those in other mammals. These observations are unlikely to be explained by unusually fast blood cell turnover, as erythrocyte half-life is assumed to be average to relatively long in nectar-feeding bats (25 to more than 100 days) [31,32].

Secondly, hyperglycaemia and acute blood glucose fluctuations may increase the proton gradient across the inner mitochondrial membrane, thereby interfering with membrane electron transfer in the respiratory chain, resulting in electron leakage and in the formation of reactive oxygen species (ROS) beyond physiologically normal levels [2,5,33,34]. This may be particularly relevant in nectar-feeding bats with their high metabolic rates, as the production of ROS correlates positively with metabolic rate [35–37].

ROS have been found to damage DNA, lipids and proteins and ROS-induced oxidative stress may be a key factor involved in the ageing process in animals, and also in the onset of the metabolic syndrome and diabetes [14,33,38]. ROS can interfere with pancreatic gene expression, lead to a dysfunction of pancreatic β-cells and prevent insulin production, but can also lead to insulin resistance in insulin-sensitive tissues [2,5,6,39]. Thus, tolerating hyperglycaemia would require bats to either be able to prevent excess production of ROS (e.g. through uncoupling proteins [14,37]), or to reduce oxidative stress, perhaps by buffering ROS with anti-oxidants [5,33,36]. Having said this, recent studies show confounding results of oxidative stress on health and ageing in mammals, which do not unequivocally support a direct connection between ROS production, oxidative damage and longevity. In some long-lived mammals (e.g. mole rats), ROS production and related damage are similar to, or even higher than, those in short-lived species [14,33]. Furthermore, some authors highlight the essential role of ROS processes such as induced stress resistance and anti-oxidant defence, which may have positive effects on lifespan [40].

A degree of tolerance of increased blood glucose might allow the bats to use the blood as a short-term glucose reservoir (e.g. to fuel high rates of glycolysis in the muscle cells during flight). Such high rates of glycolysis might also serve to reduce mitochondrial oxidation and ROS formation to levels lower than those observed in other mammals, assuming lactate produced during glycolysis could also be sufficiently rapidly eliminated to prevent blood acidosis (possibly by transamination of lactate to alanine, which does not influence blood pH). In most mammals, the increased need for glucose in contracting muscle cells during physical exercise is supplied by glycogenolysis, and overall up to 70 to 80 per cent; of the aerobic metabolism is fuelled by endogenous fuels (glycogen and triglycerides). In exercising humans, for example, dietary exogenous fuel maximally supplies 30 per cent of metabolic requirements [41,42]. It has been assumed that the proportion of exogenous substrate used is limited by the speed of intestinal carbohydrate uptake, and especially by the rate of glucose transport into muscle cells [42,43]. Nectar-feeding bats, however, have been found to fuel their energy metabolism directly and almost entirely with ingested exogenous substrates, during both rest and physical exercise [18,19]. They are clearly capable of supplying their muscle cells with recently ingested sugars in sufficient quantities to fuel

Figure 3. Blood glucose in G. soricina ingesting 2.7 g glucose kg$^{-1}$ M$^{-1}$ every 10 min. After feeding, animals were in flight for 2 min, then at rest for 8 min. Glucose was provided as a 21% sugar solution; $n = 6$. Figure 4. Blood glucose levels in G. soricina ingesting 5.4 g kg$^{-1}$ M$^{-2}$ of a 20% sugar solution (37 : 37 : 26% glucose, fructose and sucrose) every 15 min (means ± s.e.). Bats were allowed to fly for 30% (open triangles), 45% (filled circles), 60% (filled triangles) or 75% (open circles) of the 15 min time intervals between feeding; $n = 6$ per group.

Proc. R. Soc. B (2011)
their extremely high energetic needs during flight. Intestinal sugar uptake is unlikely to be a limiting factor for physical activity in nectarivorous bats, as intestinal sucrose concentrations in these bats are high [17] and sugar uptake is fast [18,19]. Sugar uptake probably takes place not only by active transport, but also via a paracellular pathway (such as has been found in frugivorous bats [44,45]), which speeds up intestinal sugar absorption significantly. In mammals, glucose uptake triggers the secretion of insulin, which increases cellular glucose uptake, mostly into the liver and fat and muscle tissues. For skeletal muscle cells, however, muscle contraction has been found to be a second principal signal pathway to increased glucose uptake. Both signal cascades independently induce the translocation of the glucose transporter protein GLUT-4 from an intracellular pool to the plasma membrane. GLUT-4 is an important transporter, crucial for glucose homeostasis, which is primarily located in the membrane of muscle and fat cells. Insulin and muscle contraction may thus have additive effects on GLUT-4 translocation and glucose uptake [43,46,47].

Interestingly, however, *G. soricina* did not sufficiently eliminate glucose from the bloodstream during rest and low levels of flight activity. It is possible that muscular enzymes (which could have eliminated glucose from the blood via glycogenesis), were saturated and other regulatory mechanisms (such as hepatic glyco- and lipogenesis) were too slow to keep up with intestinal sugar assimilation rates and prevent extreme levels of blood glucose. Instead, blood glucose increased as a function of the amount of ingested glucose. Independent of whether nectar-feeding bats have evolved a physiological tolerance for hyperglycaemia, the bats’ behaviour clearly serves to reduce the duration and magnitude of blood sugar spikes. We found that when bats spent 60 to 75 per cent of their time airborne during feeding, this balanced the rapid intestinal glucose absorption and decreased the postprandial hyperglycaemia: initial blood glucose spikes were markedly blunted (less than 14 mmol glucose l\(^{-1}\) blood) and the average blood glucose levels after 2 h were lower relative to those in bats allowed to fly less.

Field observations show that nectar-feeding bats forage for up to 12 h each night, during which feeding bouts alternate with short periods of rest [7]. *Glossophaga commissariss*, the sibling species to *G. soricina*, has been found to be active for an average 60 per cent (maximum 80%) of its time in its natural habitat at night [48], which our results suggest would serve to maintain blood glucose at normal levels during feeding.

We suggest that the evolutionary process of dietary specialization of bats on sugar-rich floral nectar was supported by a simultaneous evolution of high rates of energy expenditure, especially resulting from high flight activity, thereby protecting the bats from hyperglycaemia. The inclusion in the diet of floral nectar, a novel high-quality food source, may have enabled the bats to allocate more energy to activities with a clear selective benefit, such as searching for new roosts and food sources, or mating behaviour and other social interactions, concurrently increasing energy turnover. Alternatively, a spatially scattered distribution of floral nectar may have increased foraging costs for bats and this, in combination with resource competition with conspecifics or other nectarivores, may have resulted in the need to increase foraging effort, and thus energy expenditure. Such energetic constraints may in turn have induced adaptations to reduce flight costs, like the reduction of fat deposits and the direct fuelling of metabolic processes with exogenous fuel [18,19].

The evolution of fast glucose absorption and direct fuelling of metabolic processes may, however, also have arisen as a secondary constraint in situations when nectar is abundantly available. In the present, some of the bats’ food plants, such as *Ochroma pyramidale* and *Matisia cordata*, can produce vast amounts of nectar each night, amounting to 0.5 and 10 l of nectar, respectively [49]. An energy-efficient exploitation of such rich nectar sources would require only low flight activity, which would, however, result in hyperglycaemia. Besides evolving a tolerance for hyperglycaemia, only activity beyond that required for foraging alone would have prevented extreme blood glucose levels in such situations. Those bat individuals that, when nectar was plentiful, evolved to pursue alternative activities (such as social interactions or searching for roosts, each with its own inherent selective benefit) would have experienced less severe spikes of hyperglycaemia and related health problems, and would therefore have been the fittest. This reasoning may help to explain present-day behavioural observations of bats in captivity. Several authors have found captive nectar-feeding bats to engage in continued flight activity beyond that required for foraging, at *ad libitum* food availability, resulting in daily energy turnover similar to that of individuals in the natural habitat [7,11,12,20,50].

The mechanisms of blood glucose regulation may be similar in other nectar-feeding vertebrates with comparable lifestyles, such as honey possums or in hummingbirds. Bird and mammalian physiology are, however, likely to be different with respect to blood sugar regulation and hyperglycaemia, as avian plasma glucose concentrations are in general about two to three times those of mammals of similar *M*, so absolute blood glucose values cannot be directly compared [27,30]. However, hummingbirds show some striking similarities to nectar-feeding bats. hummingbirds are also very long-lived, have high mass-specific metabolic rates and fuel their flight costs directly with exogenous substrate [51–53]. Although these birds are mostly only active about 20 per cent of their time, they nevertheless spend most of their energy on activity [54]. Hummingbirds also experience high post-feeding blood glucose levels: in several species a fasting value of approximately 17 mmol glucose l\(^{-1}\) was shown to double after ingesting an unknown amount of glucose [30] (compared with an even more extreme four to eightfold postprandial increase in resting bats); yet these birds, like *G. soricina*, do not produce high levels of AGE (e.g. HbA1c). In general, the effects of hyperglycaemia, such as increased ROS and AGE production, are similar in birds and mammals. However, despite high metabolic rates and higher blood glucose levels, birds live longer than similar-sized mammals—a poorly understood phenomenon [14,33,51]. Comparative studies on convergently evolved nectarivorous birds and mammals on the mechanisms allowing longevity, despite an extremely sugar-rich diet, would clearly be of high interest. Such research may help to advance our understanding of both biological ageing and the development of the metabolic syndrome and diabetes [14,51].
In conclusion, nectar-feeding bats, exceptionally among mammals, may indeed have evolved mechanisms that enable them to tolerate high blood glucose levels. Independently, however, an inherent drive to engage in directed high-activity behaviours (such as foraging, searching for roosts or social interactions, each with clear selective advantages) is likely to have supported the evolutionary dietary specialization of nectar-feeding bats by suppressing post-feeding hyperglycaemia, especially when foraging on abundant, rich nectar sources. High activity would simultaneously have enhanced their efficiency as plant pollinators, because active individuals are likely to pollinate more plants and transport pollen over longer distances. From the plants’ perspective, the energetic costs of producing sugar-rich nectar would be repaid through enhanced cross-pollination.

We thank Mirkka Jones for invaluable improvements of the article; Sylvia Ortmann, Thomas H. Kunz and all anonymous reviewers for comments; and Igor Harsch and Petra Koroma for HbA1c measurements. The experiments complied with the German laws on animal experimentation and were performed under the approval of the Animal Ethics Committee of the Bavarian District Court. We dedicate this work to the memory of Otto von Helversen.

REFERENCES

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