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RESEARCH ARTICLE

Glucose tolerance of iguanas is affected by high-sugar diets in the lab and supplemental feeding by ecotourists in the wild

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ABSTRACT

There is great interspecific variation in the nutritional composition of natural diets, and the varied nutritional content is physiologically tolerated because of evolutionarily based balances between diet composition and processing ability. However, as a result of landscape change and human exposure, unnatural diets are becoming widespread among wildlife without the necessary time for evolutionary matching between the diet and its processing. We tested how a controlled, unnatural high glucose diet affects glucose tolerance using captive green iguanas, and we performed similar glucose tolerance tests on wild Northern Bahamian rock iguanas that are either frequently fed grapes by tourists or experience no such supplementation. We evaluated both short and longer-term blood glucose responses and corticosterone (CORT) concentrations as changes have been associated with altered diets. Experimental glucose supplementation in the laboratory and tourist feeding in the wild both significantly affected glucose metabolism. When iguanas received a glucose-rich diet, we found greater acute increases in blood glucose following a glucose challenge. Relative to unfed iguanas, tourist-fed iguanas had significantly lower baseline CORT, higher baseline blood glucose, and slower returns to baseline glucose levels following a glucose challenge. Therefore, unnatural consumption of high amounts of glucose alters glucose metabolism in laboratory iguanas with short-term glucose treatment and free-living iguanas exposed to long-term feeding by tourists. Based on these results and the increasing prevalence of anthropogenically altered wildlife diets, the consequences of dietary changes on glucose metabolism should be further investigated across species, as such changes in glucose metabolism have health consequences in humans (e.g. diabetes).

KEY WORDS: Glucocorticoid, *Cyclura*, *Iguana*, Glucose tolerance test, Sugar, Tourism

INTRODUCTION

Glucose, a simple sugar, represents an important source of energy for metabolism in all organisms. Glucose is highly regulated through an integrated suite of tissues and hormones (Jiang and Zhang, 2003). Disruption of glucose regulation can result in altered blood glucose levels and lead to significant health consequences (Jiang and Zhang, 2003). For example, elevated blood glucose levels have been linked to reduced offspring survival in blue tits (*Cyanistes caeruleus*) (Kaliński et al., 2014) and reduced lifespan in zebra finches (*Taeniopygia guttata*) (Montoya et al., 2018). However, blood glucose varies considerably across species, environments, timescales and sexes, and thus understanding glucose regulation in natural systems can be challenging (Gangloff et al., 2017; Kaliński et al., 2014; Montoya et al., 2018; Remage-Healey and Romero, 2000; Sandfoss et al., 2020; Sparkman et al., 2018).

Glucose tolerance tests are widely used in medical and human research as important indicators of glucose dysregulation (Andrikopoulos et al., 2008), and can also be an important tool in non-model organisms. To test glucose tolerance, a measured amount of glucose is delivered to the animal, and blood glucose levels are measured over set time intervals following delivery to determine clearance rates (Pawlak et al., 2004). Diet significantly alters glucose tolerance in well-studied animal models (Cohen and Teitelbaum, 1964; Pawlak et al., 2004), but research is limited in non-model systems regarding glucose tolerance and dietary alterations. Most work in this area is focused on human health. In humans, it is well established that a prolonged high sugar diet can ‘exhaust’ the ability to regulate blood glucose (Johnson et al., 2013; Reiser et al., 1986). These effects are compounded when combined with excess dietary fats (Boden and Laakso, 2004; Johnson et al., 2013) and even result in type 2 diabetes in mice when combined with high fat intake (Surwit et al., 1988). This is evidenced by elevated blood glucose levels (i.e. hyperglycemia; ADA, 2002). While there has been considerable investment in understanding glucose regulation in humans, little is known about the regulation of glucose or even the effects of a prolonged, unnatural high sugar diet in non-human species, with the exception of a few studies (Coulson and Hernandez, 1953; Khalil and Yanni, 1959; Montoya et al., 2020; Prado, 1946).

In addition to glucose tolerance tests, glucocorticoids may provide important information regarding glucose regulation. Glucocorticoids and the hypothalamic–pituitary–adrenal (HPA) axis are also central to the regulation of both glucose and metabolism, via promoting gluconeogenesis, regulating glycogen metabolism and even modulating pancreatic hormones that maintain glucose homeostasis in the blood (Kuo et al., 2015). For example, two separate studies in free-living gartersnakes show significant positive relationships among baseline corticosterone (CORT, the primary glucocorticoid in reptiles) and glucose, suggesting CORT is an important regulator of glucose during normal physiological function (Gangloff et al., 2017; Neuman-Lee et al., 2020). Glucocorticoids along with the sympathetic release of

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catecholamines also alter glucose trafficking and metabolism in response to perturbations that elicit stress in organisms (Romero and Butler, 2007; Saplosky, 1992; Sapolsky et al., 2002). Therefore, investigating glucocorticoids in addition to glucose tolerance may better inform the effects of dietary changes on glucose regulation.

Supplemental feeding of wildlife offers an opportunity to investigate the effects of altered diet and energetic state on the physiology of free-living organisms, whereby it often introduces non-natural food items and thus affects animal nutrition and energy balance (French et al., 2022; Stewart et al., 2016). For example, supplemental feeding of green turtles (*Chelonia mydas*) in the Canary Islands is leading to changes in biochemical markers in the blood that are linked to high consumption of proteins and fat (Monzón-Argüello et al., 2018). In the long-term, diet modifications could impact nutrient processing, especially when supplemented food does not have a nutritional make-up similar to that of the natural diet to which their digestive system has evolved.

The northern Bahamian rock iguana (*Cyclura cychlura*) occurs in the Exuma island chain of The Bahamas, is subject to these tourist feeding activities, and provides an excellent natural experiment to study the effects of supplemental feeding of wildlife in nature. Many visitors to The Bahamas take day boating trips to visit and feed these iguanas. Feeding by tourists leads to unnatural aggregations of iguanas and diet alterations to a large proportion of an island's population (Hines, 2011; Iverson et al., 2006). Grapes, the most common food fed to the iguanas, are high in sugar, both glucose and fructose (Muñoz-Robredo et al., 2011), so animals at tourist-visited sites are receiving regular sugar supplementation. Some islands are regularly visited by hundreds of tourists daily while adjacent islands receive no visitors, providing an excellent opportunity for examining the impact of supplemented diets through comparisons of multiple fed and non-fed populations. Moreover, the ecology and ecophysiology of the species has been studied extensively, providing a valuable foundation for a specific study on sugar metabolism. In addition, the ability to experimentally test the effects of regular glucose feeding on glucose tolerance in a controlled laboratory setting using captive-bred green iguanas (*Iguana iguana*) can provide complementary data to those obtained under more natural but less controlled field conditions.

In this study, we conducted glucose tolerance tests in three separate iterations: (1) effects of supplemental glucose on blood glucose regulation in captive green iguanas; (2) effects of ecotourism and associated feeding of unnatural foods including grapes on blood glucose regulation in Northern Bahamian rock iguanas using a blood sampling frequency designed to assess a broader span of the glucose processing time (i.e. 19 h); and (3) a similar study in these iguanas focused on the initial post-challenge increase in blood glucose (i.e. 8 h). We thus experimentally tested the effects of sugar supplementation under controlled conditions as well as in a real-world environment. In the field, we simultaneously measured CORT and glucose to look at potential interactions. By conducting multiple studies using two species and two experimental settings, we were able to better evaluate the reliability of our findings. We tested the hypothesis that prolonged unnatural levels of dietary glucose leads to changes in glucose metabolism, akin to the response in humans. We predicted that lab-based experimental supplementation in green iguanas and tourist feeding of grapes to free-ranging rock iguanas would both lead to glucose responses that are indicative of glucose intolerance, whereby blood glucose would be elevated at baseline, as well as peak higher and return more slowly to baseline following a glucose challenge. Previous work (French et al., 2022) demonstrated that baseline CORT is significantly lower at tourist sites. However, we predicted that animals fed by tourists would show greater increases in CORT following glucose tolerance tests, akin to what is expected in blood glucose responses (Pawlak et al., 2004).

MATERIALS AND METHODS

Study system

Northern Bahamian rock iguanas are large-bodied, long-lived herbivorous lizards native to Andros Island and about a dozen small islands of the Exuma island chain in The Bahamas. *Cyclura cychlura inornata* (Barbour & Noble 1916) is native to several cays in the northern Exumas and *C. c. figginsi* (Barbour 1923) inhabits a handful of cays in the southern Exumas (Malone et al., 2003). Both subspecies are listed under Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Iverson et al., 2019; Knapp and Buckner, 2004). Despite this protection, Northern Bahamian rock iguanas are a significant component of Bahamian ecotourism, with both private and commercial boats bringing tourists nearly every day to visit populations of rock iguanas. An essential part of the tourist experience in the Exumas has become feeding the iguanas grapes served on wooden skewers. Because accessibility varies among the iguana-inhabited islands, some of the populations receive hundreds of tourists each day while adjacent, biogeographically similar islands rarely, if ever, see tourists. This varied visitation provides an ideal natural experiment to study the impact of human disturbance and altered diet on physiology.

Rock iguanas present at the feeding beaches are larger, grow faster and weigh more (relative to body length) compared with iguanas that do not receive visitors (Smith and Iverson, 2016). However, iguanas at feeding beaches also have looser feces, higher endoparasitic infection rates and altered plasma levels of diet-related compounds, all of which may reflect the unnatural diet that the iguanas are receiving (Knapp et al., 2013).

Because of the unnaturally high sugar content of a diet that consists of daily consumption of grapes, we examined whether ecotourism, and presumably the supplemental feeding of grapes, alters blood glucose regulation. We conducted two glucose tolerance experiments on free-ranging northern Bahamian rock iguanas, each using two population pairs of adjacent islands within the Exuma island chain. Within each island pair, one island is visited by large numbers of tourists while the other rarely receives human visitors.

As the field experiment could not isolate the effects of the high sugar diet supplementation from other aspects of the ecotourism visits, we also conducted a laboratory-based study where glucose intake was the only variable that differed between two treatment groups. Owing to the protected status of the rock iguanas, we could not use this species for the laboratory experiment. Instead, we used juvenile green iguanas [*Iguana iguana* (Linnaeus 1758)]. Green iguanas are also large-bodied herbivorous lizards, but have a more tropical distribution, widely distributed throughout the tropics of Latin America. Relatively recently, green iguanas have been introduced into Florida, USA, some of the Caribbean islands, and many other locations around the world (Bock et al., 2016). Rock iguanas were handled and blood samples collected and exported with appropriate Utah State University Institutional Animal Care and Use Committee (IACUC) approval (no. 2530), research permissions from The Bahamas Environment, Science & Technology Commission (to C.R.K. and J.B.I.), and CITES export permits from The Bahamas Department of Agriculture (to C.R.K.). The green iguana laboratory study was conducted with Arizona State University IACUC approval (no. 14-1340R).

Study 1: effects of supplemental blood glucose on blood glucose regulation in captive green iguanas using a 49 h glucose tolerance test

Twenty-four juvenile green iguanas were acquired from Backwater Reptiles (Rocklin, CA, USA) in April 2016. The iguanas were housed singly in standard rodent cages (47.6×26.0×20.3 cm, depth×width×height) with custom, fine-screen metal lids. Each cage was equipped with a sheet of Techboard paper (Shepherd Specialty Products, Watertown, TN, USA) on the cage floor, a water bowl, and a wooden perch angled at ∼45 deg under a 25 W incandescent light bulb at one end of the cage to provide a basking site and thermogradient. The iguana cages were also lightly sprayed with water daily as iguanas sometimes prefer to drink water droplets from surfaces rather than from a water bowl. Room temperature was maintained at 25.5°C and both room and cage lights came on at 07:00 h and went off at 19:00 h. Iguanas were fed daily a combination of spring mix leafy greens and moistened Tortoise Diet (Mazuri, St Louis, MO, USA).

After acclimating for 14 days, animals were weighed using a platform scale (±0.1 g). Four days later, in addition to their regular diet, the green iguanas began receiving an oral gavage every other day of either water (control group), glucose at a dose of 2.5 g $\mathrm{kg^{-1}}$ body mass (low dose) or glucose at a dose of 5.0 g $\mathrm{kg^{-1}}$ (high dose). Iguanas were assigned to the three treatments via a mixed interspersion design whereby animals were placed in groups sequentially based on body mass to balance sizes among groups (ANOVA testing for body mass differences according to treatment, $F_{2,2}=0.046$, $P=0.955$).

Glucose doses were based on observations of grapes consumed by rock iguanas on tourist-visited islands (6 but often more per individual per day, personal observation) and the amount of glucose and total sugars present in Thompson seedless grapes (8.71 and 17.67 g per 100 g of grapes, respectively; Muñoz-Robredo et al., 2011), assuming an average grape weighs 4.9 g (USDA National Nutrient Database for Standard Reference, release 28 May 2016). The low dose provides glucose in an amount similar to that found in consumed grapes, while the high dose approximates the amount of total sugars in consumed grapes. Glucose solutions were made by pulverizing 1 g glucose tablets (CVS Health Glucose Tablets Grape, Item no. 235152) and then dissolving the glucose in water to create a 0.5 g $\mathrm{ml^{-1}}$ glucose slurry. Captive green iguanas were gavaged every other day to limit handling stress, and they were gavaged a total of nine times over 17 days.

Following 17 days of glucose treatment every other day, all animals were given a glucose tolerance test. Based on published intestinal clearance time in green iguanas (Smith et al., 2001), we eliminated the potential influence of ingested food on blood glucose concentrations by fasting the iguanas starting a day prior to the onset and throughout the duration of the glucose tolerance test. Initial blood glucose concentrations were determined pre-challenge by collecting a droplet of blood from the caudal vein of each iguana using a 1 ml syringe with a 25-gauge X 3/8″ needle. Immediately after the initial blood sample, each iguana received a glucose challenge using an intermediate glucose dose (3.75 g glucose $\mathrm{kg^{-1}}$). Additional blood glucose determinations were made at 1, 3, 11, 25 and 49 h post glucose challenge, with each captive iguana being returned to its home cage between sampling time points. An end body mass was obtained after the 49 h blood sampling. Blood samples were collected within 3 min of removing the iguana from its cage, and blood glucose was immediately determined from the whole blood using a handheld glucometer (EvenCare G2 Blood Glucose Monitor, no. MPH1540).

Study 2: effects of ecotourism and associated grape feeding on blood glucose regulation in northern Bahamian rock iguanas using a 19 h glucose tolerance test

In June 2016, 12 adult northern Bahamian rock iguanas were captured on each of four islands of the Exuma island chain. Two of the islands, Allen's Leaf Cay and Flat Rock Reef Cay, are approximately 1 km from each other at the northern end of the Exumas and are inhabited by the Allen Cays rock iguana (*C. c. inornata*). Allen's Leaf Cay is heavily visited by commercial ecotourism boats, bringing 200+ grape-feeding ecotourists to the island per day (Smith and Iverson, 2016). In contrast, Flat Rock Reef Cay is very difficult to access and therefore rarely has any human visitors and the iguanas there virtually never receive food from humans.

The other two islands, Leaf Cay and North Adderly Cay, are approximately 0.7 km from each other at the southern end of the Exumas and are inhabited by the Exuma Island rock iguana (*C. c. figginsi*). Like Allen's Leaf Cay in the north, Leaf Cay is heavily visited by grape-feeding ecotourists while North Adderly Cay rarely has any human visitors, much like Flat Rock Reef Cay in the north.

The iguana populations on all four of these islands have been part of long-term biological studies (Iverson et al., 2006; Knapp et al., 2013, 2019; Smith and Iverson, 2016). Iguanas were captured using large handheld fish landing nets. For the tourist-visited islands, all iguanas were captured before the arrival of the first tourist boats of the day to ensure that the iguanas had not recently consumed grapes. Immediately upon capture, a 1 ml blood sample was collected from the iguana's caudal vein for a baseline blood glucose concentration for this study and for assessments related to other studies. Samples were collected between 08:00 h and 13:00 h to reduce daily fluctuations and within 3 min of capture and handling. There was no effect of time of day or capture and handling duration on CORT or blood glucose (all $F<1.336$, all $P>0.608$). Each iguana was then put in its own large cloth bag that was placed in the shade until further processing.

Shortly after capture, the bagged iguana was weighed using a spring scale (±5.0 g; Pesola, Schindellegi, Switzerland), sexed and orally gavaged with glucose using the same dose as used for the green iguanas (3.75 g $\mathrm{kg^{-1}}$). Each iguana was bled 3, 11 and 19 h post-gavage and held in the cloth bag between bleeds. Between the 11 and 19 h blood collections, the bagged iguanas were moved from the island to our support boat for logistical reasons. After completion of the glucose tolerance test, the iguanas were further processed for another study and then returned to their sites of capture the next morning. Unfortunately, owing to logistical limitations there was insufficient time and iguanas (for some islands) to include no-glucose gavage control groups at each island for either study 2 or 3. Instead, to test for the effects of gavage we reference our controlled laboratory study. Moreover, previous studies performing glucose tolerance test in reptiles and birds demonstrated significantly elevated levels of blood glucose in animals given glucose relative to saline controls (Montoya et al., 2020; Prado, 1946).

Study 3: effects of ecotourism and associated grape feeding on initial blood glucose response in northern Bahamian rock iguanas using an 8 h glucose tolerance test

In June 2018, we conducted a study similar to study 2, except that we used Noddy Cay, instead of North Adderly Cay, for the non-visited island in the southern Exumas and blood samples were collected at 0, 1, 3, 5 and 8 h to better examine the early response to the glucose challenge. This study enabled us to sample more frequently during the time frame when the largest differences among groups were observed in earlier studies. We were thus better able to investigate the rate of glucose increase and timing of glucose peak levels. Sample sizes were 20, 15, 15 and 15 adult iguanas for Allen's Leaf, Flat Rock Reef, Leaf and Noddy Cays, respectively. Because of the shorter duration of the study, all samples were collected on the islands, negating any need to transport the iguanas to the boat. After completion of the glucose tolerance test, the iguanas were further processed for another study and then returned to near their sites of capture. As with the 2016 study, samples were collected between 08:00 h and 13:00 h to reduce daily fluctuations and within 3 min of capture and handling. There was also no effect of time of day or capture and handling duration on CORT or blood glucose (all $F<2.092$, all $P>0.153$). Owing to logistical limitations and weather conditions, we were unable to collect the 8 h blood samples for the last 4 animals captured on Flat Rock Reef Cay.

Radioimmunoassay

Circulating CORT reactivity to the capture, glucose challenge and confinement was measured in the rock iguanas by analyzing the differences from baseline (0) to either 3 h (2016) or 1 h (2018) samples. Concentrations of CORT were determined using a radioimmunoassay protocol (French et al., 2010). Briefly, plasma samples were extracted using a solution of 30% ethyl acetate: isooctane and assayed in duplicate for CORT (Ab: catalog no. 07120016, diluted 1:100, MP Biomedicals). Final concentrations were calculated by averaging the duplicate samples and adjusted for accuracy using individual recoveries. Intra-assay coefficients of variation were 14.00% (study 1 and 2 samples were run in the same assay) and 11.50% (study 3).

Statistical approaches

Using R statistical software (v. 3.5.1; https://www.r-project.org/), we built mixed models with the package 'nlme' (v. 3.1.142; https://CRAN.R-project.org/package=nlme) to test glucose responses from each study. Candidate models consisted of additive and interactive combinations of fixed-effect parameters. For study 1, this included time (number of hours), treatment (control, low or high) and body mass. For studies 2 and 3, we considered time (number of hours), tourism (presence or absence), subspecies (*C. c. figginsi* or *C. c. inornata*), sex (male or female), time of capture, and body condition and mass (see below). All models included a random intercept of individual identity. Prior to model construction, we $\log_e$-transformed glucose values to improve the distribution of model error terms and random effects. For study 1, we found one model with the interaction of time and treatment as fixed effects and identity as a random intercept to best fit the data (Δ_i=0.00, W_i=1.0; Table 1). For studies 2 and 3, we found one model with the interaction of time, tourism and subspecies as fixed effects and identity as a random intercept to best fit the data (Δ_i=0.00, W_i=1.0; Table 1).

With the package 'AICcmodavg' (v. 2.2.2; https://CRAN.R-project.org/package=AICcmodavg), we used likelihood ratio tests to compare models and implement model selection based on second order Akaike's information criterion (AICc) scores (Akaike, 1974, 1998; Hurvich and Tsai, 1991; Sugiura, 1978). While considering models with low delta AICc scores (Δ_i<2) and proportionate Akaike weights (W_i), we selected those more inclusive to interpreting the physiological basis of the response (Aho et al., 2014; Burnham and Anderson, 2002, 2004). We performed marginal type III sum of squares tests to determine significance for the fixed effects in each of our selected models. Model summary outputs provided us with estimates of beta coefficients with 95% confidence intervals (CI) and *P*-values for each fixed-effect parameter as well as the variance (σ^2) with 95% CI for the random intercept. If fixed effects were significant in a model, we used the package 'emmeans' (v. 1.4.7; https://CRAN.R-project.org/package=emmeans) to acquire estimated marginal means and test level differences with Tukey-adjusted contrasts.

At baseline, peak (3 h in study 1 and 2; 5 h in study 3) and end-time points, we performed Student's *t* or Tukey's HSD pairwise *post hoc* comparisons where appropriate (adjusted alpha level for multiple comparisons), to test for specific group differences among glucose treatments in the laboratory, and tourism and subspecies in the field. For study 2, we were required to use nonparametric tests (Wilcoxon signed rank) owing to the non-normal distribution of the data that were not readily transformable. Although the peak for blood glucose in study 3 was at 5 h, the results were the same if analyzed at 3 h for consistency with the other two studies.

We used separate two-way repeated measures ANOVAs to test for the effects of tourism and subspecies on CORT concentrations over time for studies 2 and 3 in the field. Within subject comparisons in the repeated measures analysis that violated the assumptions of sphericity were Greenhouse–Geisser (G-G) corrected. We included body condition (calculated as the residuals resulting from the regression of snout vent length and body mass) as a covariate in the ANOVAs for studies 2 and 3. We also initially tested body mass as a covariate but the results did not differ significantly from body condition. For all studies we found no apparent sex differences in CORT (with the exception of 2018 CORT concentrations) and thus sexes were combined for all analyses (all $F<0.047$, all $P>0.830$). For 2018 CORT analyses we did not have sufficient statistical power given our sample size to investigate sex as a factor along with

Table 1. Model selection table for top three models fitting glucose as a response variable in juvenile green iguanas (study 1) and adult northern Bahamian rock iguanas (studies 2 and 3)

	Model	log Likelihood	AICc	Delta AICc (Δ_i)	Akaike weight (W_i)
Study 1	**Glucose∼Time×Treatment+(1 | Identity)**	**1.59**	**43.66**	**0.00**	**1.00**
	Glucose∼1+(1 | Identity)	−62.76	131.69	88.04	0.00
	Glucose∼Time×Treatment×Body Mass+(1 | Identity)	−83.80	271.82	228.17	0.00
Study 2	**Glucose∼Time×Tourism×Subspecies+(1 | Identity)**	**−39.91**	**120.17**	**0.00**	**1.00**
	Glucose∼1+(1 | Identity)	−125.15	256.43	136.26	0.00
	Glucose∼Time×Tourism×Subspecies×Body Condition+ (1 | Identity)	−124.15	333.17	213.00	0.00
Study 3	**Glucose∼Time×Tourism×Subspecies+(1 | Identity)**	**−26.10**	**101.08**	**0.00**	**1.00**
	Glucose∼1+(1 | Identity)	−180.18	266.46	265.38	0.00
	Glucose∼Time×Tourism×Subspecies×Body condition+ (1 | Identity)	−135.92	366.46	265.38	0.00

Bold indicates the model selected for analyzing glucose in each study.

tourism (i.e. tourism or no tourism) and subspecies (i.e. *inornata* or *figginsi*), but sex ratios were balanced across groups.

Finally, correlations between glucose and CORT at baseline and 3 h (2016) or 1 h (2018) were conducted using bivariate analyses. CORT repeated measures ANOVAs and bivariate analyses of CORT and glucose were conducted using JMP 12.1.0 (SAS Institute, Inc.).

RESULTS

Glucose

Study 1: effects of supplemental glucose on blood glucose regulation in green iguanas using a 49 h glucose tolerance test

The number of hours after glucose treatment (Time) corresponded with significant increases in blood glucose, whereby peak levels increased 137% from baseline at 3 h ($P<0.001$; Tables 2 and 3; Table S1; Fig. 1). Treatment did not have an overall significant effect on glucose levels ($P=0.650$), nor did the interaction of treatment and time ($P=0.336$). Other fixed effects were not directly tested as they were not likely to add significant information based on model selection (Table 1).

Post hoc comparisons at 3 h peak glucose levels for ‘high’ treatment animals were significantly different from control animals ($t=2.385$, $P=0.027$), and ‘low’ treatment animals were intermediate to the other two groups (all $t<-1.580$, all $P>0.129$). There were no treatment differences at baseline (0 h) or end time (49 h) (all $t<-1.623$, all $P>0.120$).

Study 2: effects of ecotourism and associated grape feeding on blood glucose regulation in northern Bahamian rock iguanas using a 19 h glucose tolerance test

The number of hours after glucose treatment (Time) corresponded with significant increases in blood glucose, whereby peak levels increased 167% from baseline at 3 h ($P<0.001$; Tables 2 and 4; Table S1; Fig. 2). Tourism did not have a significant effect on overall glucose levels ($P=0.261$), nor did subspecies ($P=0.991$). The interaction of tourism and time was marginally significant ($P=0.055$), and all other interactions were not significant ($P>0.05$). Other fixed effects were not directly tested as they were not likely to add significant information (Table 1).

Table 2. Type III sums of squares for mixed models of glucose responses in juvenile green iguanas (study 1) and adult northern Bahamian rock iguanas (study 2 and 3)

	Fixed effects	χ^2	d.f.	*P*
Study 1	Time	95.592	5	**<0.001**
	Treatment	0.861	2	0.650
	Time×Treatment	11.281	10	0.336
Study 2	Time	113.154	3	**<0.001**
	Tourism	1.266	1	0.261
	Subspecies	0.0001	1	0.991
	Time×Tourism	7.5879	3	0.055
	Time×Subspecies	3.1518	3	0.369
	Tourism×Subspecies	0.1547	1	0.694
	Time×Tourism×Subspecies	3.8363	3	0.280
Study 3	Time	201.310	4	**<0.001**
	Tourism	20.898	1	**<0.001**
	Subspecies	6.974	1	**0.008**
	Time×Tourism	2.398	4	0.663
	Time×Subspecies	10.695	4	**0.030**
	Tourism×Subspecies	9.867	1	**0.002**
	Time×Tourism×Subspecies	4.604	4	0.330

Model terms include interactive combinations of fixed effects (time, tourism and subspecies). Bold *P*-values indicate significance (alpha level of 0.05).

Table 3. Mixed model summary for the effects of supplemental dietary glucose on blood glucose levels over 49 h in juvenile green iguanas using a glucose tolerance test

Fixed effects	β (95% CI)	*P*
Time (hour 1)	1.738 (1.442–2.094)	**<0.001**
Time (hour 3)	1.492 (1.238–1.798)	**<0.001**
Time (hour 11)	1.326 (1.101–1.598)	**<0.001**
Time (hour 25)	2.073 (1.720–2.497)	**0.003**
Time (hour 49)	1.018 (0.845–1.226)	0.851
Treatment (low)	0.960 (0.766–1.202)	0.367
Treatment (high)	0.905 (0.722–1.134)	0.707
Time (hour 1)×Treatment (low)	1.062 (0.816–1.383)	0.694
Time (hour 3)×Treatment (low)	1.239 (0.952–1.612)	0.209
Time (hour 11)×Treatment (low)	1.023 (0.786–1.331)	0.524
Time (hour 25)×Treatment (low)	1.273 (0.978–1.657)	0.962
Time (hour 49)×Treatment (low)	1.150 (0.883–1.496)	0.749
Time (hour 1)×Treatment (high)	1.054 (0.810–1.372)	0.65
Time (hour 3)×Treatment (high)	0.919 (0.706–1.196)	0.072
Time (hour 11)×Treatment (high)	1.006 (0.773–1.310)	0.11
Time (hour 25)×Treatment (high)	1.183 (0.909–1.540)	0.865
Time (hour 49)×Treatment (high)	1.044 (0.802–1.358)	0.296

Included are back-transformed beta coefficient estimates, and *P*-values for each fixed effect (time and treatment); bold *P*-values indicate significance (alpha level of 0.05). Random intercept of identity σ^2 (95% CI)**=**1.207 (1.178–1.24).

Post hoc comparisons revealed that tourist-fed iguanas had higher blood glucose levels at 0 h (baseline), 3 h (peak) and 19 h (end time; *post hoc* Student’s *t* adjusted values for multiple comparisons; all $t>-2.27$, all $P<0.028$). There were no significant effects of subspecies, body condition or other interactions.

Study 3: effects of ecotourism and associated grape feeding on initial blood glucose response in rock iguanas using an 8 h glucose tolerance test

The number of hours after glucose treatment (time) corresponded with significant increases in blood glucose, whereby peak levels increased 208% from baseline at 5 h ($P<0.001$; Tables 2 and 5; Table S1; Fig. 3). Overall, glucose levels were significantly higher at

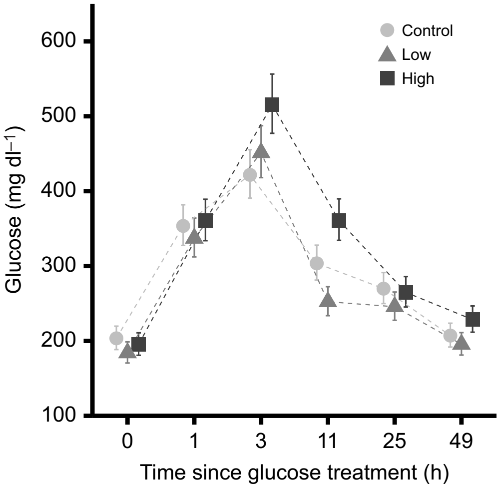

Fig. 1. Longitudinal comparisons of blood glucose concentrations (mg dl^{-1}) over 49 h following a glucose tolerance test in laboratory housed green iguanas given a 17 day supplemental glucose treatment. Control, water; low, 2.5 g glucose kg^{-1} body mass; high, 5.0 g glucose kg^{-1} body mass. Points with error bars represent back-transformed estimated marginal means (±s.e.m.) from a fitted mixed model. These data were gathered by using individuals as replicates in a lab setting. *N*=8 per group.

Table 4. Mixed model summary for the effects of ecotourism and associated tourist feeding on blood glucose levels in northern Bahamian rock iguanas over 19 h using a glucose tolerance test

Fixed effects	β (95% CI)	P
Time (hour 3)	2.662 (2.128–3.330)	**<0.001**
Time (hour 11)	2.601 (2.080–3.254)	**<0.001**
Time (hour 19)	1.326 (1.060–1.659)	**0.014**
Tourism (presence)	1.149 (0.895–1.474)	0.267
Subspecies (*C. c. inornata*)	1.001 (0.780–1.285)	0.991
Time (hour 3)×Tourism	1.083 (0.795–1.476)	0.61
Time (hour 11)×Tourism	0.933 (0.685–1.271)	0.656
Time (hour 19)×Tourism	1.395 (1.024–1.900)	**0.035**
Time (hour 3)×Subspecies	0.934 (0.686–1.273)	0.664
Time (hour 11)×Subspecies	0.851 (0.624–1.159)	0.302
Time (hour 19)×Subspecies	1.113 (0.817–1.516)	0.495
Tourism×Subspecies	0.935 (0.662–1.320)	0.696
Time (hour 3)×Tourism×Subspecies	0.950 (0.619–1.457)	0.359
Time (hour 11)×Tourism×Subspecies	1.22 (0.795–1.871)	0.309
Time (hour 19)×Tourism×Subspecies	0.802 (0.523–1.23)	0.813

Included are back-transformed beta coefficient estimates, and P-values for each fixed effect (time, tourism, and subspecies). Bold P-values indicate significance (alpha level of 0.05). Random intercept of identity σ^2 (95% CI)**=**1.288 (1.249–1.332).

tourist sites (P<0.001) and for *C. c. figginsi* (P=0.008). The interaction of time and subspecies was significant for glucose (P=0.03), such that overall levels were higher for *C. c. figginsi* by 1 h (P=0.024). The interaction of tourism and subspecies was also significant for glucose (P=0.002). Here, glucose levels were higher at visited sites for both subspecies (P<0.001). Further, tourist populations of *C. c. inornata* had higher glucose than non-visited *C. c. figginsi* populations (P<0.001), and tourist *C. c. figginsi* populations had higher glucose than non-visited *C. c. inornata* populations (P<0.001). Glucose levels for tourist *C. c. inornata* populations were lower than tourist *C. c. figginsi* populations (P=0.003), while levels did not differ between non-visited populations of each subspecies (P=0.796). All other interactions were not significant (P>0.05). Other fixed effects were not directly tested as they were not likely to add significant information (Table 1).

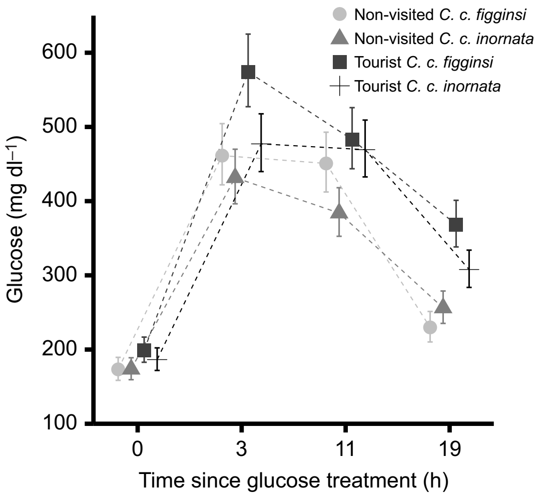

Fig. 2. Longitudinal comparisons of blood glucose concentrations over 19 h following a glucose tolerance test on in two subspecies of northern Bahamian rock iguanas at either visited or non-visited sites. Points with error bars represent back-transformed estimated marginal means (±s.e.m.) from a fitted mixed model. These data were gathered by using individuals as replicates in a field setting. N=12 per group.

Table 5. Mixed model summary for the effects of ecotourism and associated tourist feeding on blood glucose levels in northern Bahamian rock iguanas over 8 h using a glucose tolerance test

Fixed effects	β (95% CI)	P
Time (hour 1)	2.254 (1.826–2.783)	**<0.001**
Time (hour 3)	3.113 (2.522–3.844)	**<0.001**
Time (hour 5)	3.362 (2.723–4.151)	**<0.001**
Time (hour 8)	3.750 (3.038–4.630)	**<0.001**
Tourism (presence)	1.630 (1.320–2.012)	**<0.001**
Subspecies (*C. c. inornata*)	1.326 (1.074–1.637)	**0.009**
Time (hour 1)×Tourism	0.944 (0.701–1.272)	0.703
Time (hour 3)×Tourism	0.887 (0.658–1.194)	0.426
Time (hour 5)×Tourism	0.905 (0.672–1.219)	0.510
Time (hour 8)×Tourism	0.800 (0.594–1.078)	0.141
Time (hour 1)×Subspecies	0.618 (0.458–0.832)	**0.002**
Time (hour 3)×Subspecies	0.769 (0.571–1.036)	0.084
Time (hour 5)×Subspecies	0.84 (0.624–1.132)	0.251
Time (hour 8)×Subspecies	0.761 (0.565–1.025)	0.072
Tourism×Subspecies	0.636 (0.478–0.845)	**0.002**
Time (hour 1)×Tourism×Subspecies	1.427 (0.956–2.132)	0.082
Time (hour 3)×Tourism×Subspecies	1.339 (0.896–1.999)	0.153
Time (hour 5)×Tourism×Subspecies	1.241 (0.831–1.854)	0.289
Time (hour 8)×Tourism×Subspecies	1.472 (0.986–2.199)	0.059

Included are back-transformed beta coefficient estimates, and P-values for each fixed effect (time, tourism and subspecies). Bold P-values indicate significance (alpha level of 0.05). Random intercept of identity σ^2 (95% CI)= 1.27 (1.242–1.302).

Separate nonparametric analyses revealed that blood glucose differed significantly according to tourism status at 0, 5 and 8 h, with iguanas at tourist sites having higher baseline glucose (Wilcoxon; all S<723.5; Z>−3.501, P>z=0.001). Baseline blood glucose also differed by subspecies (Wilcoxon; S=837.5; Z=−2.000; P>z=0.046) with *C. c. inornata* generally exhibiting higher levels compared with *C. c. figginsi*.

Corticosterone

Study 2: effects of ecotourism and associated grape feeding on blood glucose regulation in rock iguanas using a 19 h glucose tolerance test

Time, tourism status and their interaction were significant when comparing change in CORT from 0 to 3 h, such that iguanas from

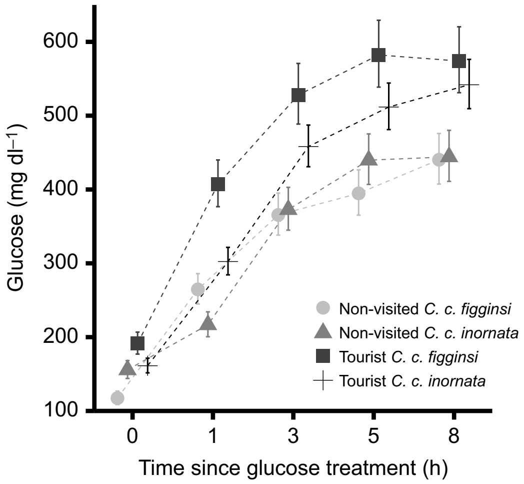

Fig. 3. Longitudinal comparisons of blood glucose concentrations over 8 h following a glucose tolerance test in two subspecies of northern Bahamian rock iguanas at either visited or non-visited sites. Points with error bars represent back-transformed estimated marginal means (±s.e.m.) from a fitted mixed model. These data were gathered by using individuals as replicates in a field setting. N=15 per group, except 20 opportunistically at Tourist *C. c. inornata*.

Table 6. Study 2: 2016 repeated measures ANOVA statistics summary of CORT from 0 h to 3 h following a glucose tolerance test

Fixed effects	F	d.f.	P
Overall model	4.683	4,40	**0.003***
Tourism	14.041	1,40	**<0.001***
Subspecies	1.089	1,40	0.303
Tourism×Subspecies	2.868	1,40	0.098
Body condition	0.043	1,40	0.837
Time (G-G corrected)	100.033	1,40	**<0.001***
Time×Tourism (G-G corrected)	9.457	1,40	**0.004***
Time×Subspecies (G-G corrected)	0.065	1,40	0.800
Time×Tourism×Subspecies	1.646	1,40	0.207
Time×Body condition	0.006	1,40	0.937

Within-subject comparisons in the repeated measures analysis violated the assumptions of sphericity and were Greenhouse–Geisser (G-G) corrected. Bold *P*-values indicate significance (alpha level of 0.05).

tourist sites exhibited lower baseline CORT levels but showed steeper increases in response to the activities associated with the glucose tolerance test (rmANOVA; Table 6; Table S2). Body condition and other interactions were not significant. *Post hoc* comparisons revealed that iguanas from tourist sites exhibited lower baseline CORT than non-tourist iguanas (*post hoc* Student's t=4.04, P<0.001). There was no relationship between CORT and blood glucose levels at 0 h or 3 h (all F<0.521, all P>0.474).

Study 3: effects of ecotourism and associated grape feeding on initial blood glucose response in rock iguanas using an 8 h glucose tolerance test

One *C. c. inornata* CORT sample was excluded as it was more than two standard deviations above the mean, constituting an extreme outlier. There was an effect of Tourism status×Subspecies, Time, Time×Tourism status and Time×Subspecies on change in CORT from 0 h to 1 h (rmANOVA; Table 7; Table S2), but not of Body condition or other interactions. *Post hoc* comparisons revealed that *C. c. inornata* from non-visited sites had higher 0 h baseline CORT levels than all other groups (Tukey's HSD, all P<0.004), but that *C. c. figginsi* from tourist sites had the highest levels at 1 h (Tukey's HSD, all P<0.015), suggesting that tourist *C. c. figginsi* had the greatest CORT response. Bivariate analyses comparing CORT with glucose at 1 h within tourist iguanas showed a significant positive relationship (Fig. S1A; $F_{1,32}$=7.724, P=0.009; positive rsq adj=0.169), but not in tourist iguanas at 0 h (Fig. S1B; $F_{1,32}$=0.664, P=0.421; positive rsq adj=−0.0103). There was no significant relationship between CORT and glucose at 1 h or 0 h in non-tourist iguanas (all F<3.349, all P>0.078).

Table 7. Study 3: 2018 repeated measures ANOVA statistics summary of CORT from 0 h to 1 h following a glucose tolerance test

Fixed effects	F	d.f.	P
Overall model	4.919	4,55	**<0.001***
Tourist	0.008	1,55	0.927
Subspecies	0.031	1,55	0.862
Tourism×Subspecies	29.351	1,55	**<0.001***
Body condition	1.225	1,55	0.235
Time	93.196	1,55	**<0.001***
Time×Tourism	15.990	1,55	**<0.001***
Time×Subspecies	8.868	1,55	**0.004***
Time×Tourism×Subspecies	0.002	1,55	0.967
Time×Body condition	0.742	1,55	0.393

Bold *P*-values indicate significance (alpha level of 0.05).

DISCUSSION

Overall, we found significant effects of both experimental sugar supplementation in the laboratory and tourist feeding in the wild on glucose tolerance and metabolism in both species of iguanas. Specifically, in the free-ranging rock iguanas, we saw significantly elevated baseline levels as well as more rapid elevation, higher peak and slower declines in blood glucose levels following a glucose challenge. We also found a greater increase in blood glucose initially following a glucose challenge in the laboratory-based green iguanas. Thus, rock iguanas chronically visited by tourists exhibited altered glucose tolerance relative to non-visited iguanas, while the 17-day glucose-supplemented green iguanas exhibited a similar but less-pronounced effect. Future work is needed to determine whether altered glucose metabolism is detrimental to iguana health.

Laboratory green iguanas

Even relatively short-term exposure (17 days) to elevated dietary glucose had significant effects on glucose tolerance, such that glucose concentrations differed according to treatment over time. Specifically, animals treated with high but ecotourism-relevant doses of sugar showed a greater blood glucose peak at 3 h post-glucose challenge. The spike at 3 h is similar to what we found in the free-living rock iguanas. Glucose clearance was much slower than that seen in mammals (Andrikopoulos et al., 2008), but other reptilian species undergoing glucose tolerance tests exhibited similar responses to that of the iguanas (Coulson and Hernandez, 1953; Khalil and Yanni, 1959; Prado, 1946), although the effects of diet on glucose tolerance were not examined. In other species, high fat diets led to glucose intolerance, including certain strains of lab mice (C57BL/6J) on high fat/simple carbohydrate diets and tilapia (*Oreochromis niloticus*) on high lipid diets (Du et al., 2020; Surwit et al., 1988).

In the captive green iguanas, unlike free-living rock iguanas, there was no significant difference among treatments in baseline blood glucose or at time points other than the 3 h glucose peak. This difference between captive and field iguanas may be due to the fact that laboratory animals were treated with glucose for a relatively short amount of time when compared with animals in the field that have been experiencing feeding by tourists daily for years. Thus, the less-pronounced glucose tolerance responses to high glucose diets in the lab may reflect an early disturbance in glucose metabolism. However, the laboratory animals were juveniles, whereas field animals were adults, and this could lead to differences in glucose tolerance as could other differences between the laboratory and field studies, including the use of repeated gavaging in the laboratory to deliver the glucose, species-specific differences and effects of captivity.

Baseline levels in free-ranging rock iguanas

Unlike the relatively short-term laboratory study, the free-living rock iguanas on tourist-visited islands have been supplementally fed on a near daily basis for many years, resulting in a 'natural' chronic treatment. In both the 8 h and 19 h response studies, iguanas on the tourist-visited islands had higher blood glucose and lower CORT at baseline relative to iguanas on unvisited islands. The elevation in blood glucose is unsurprising, given that previous work with this species also found that food supplementation resulted in elevated blood glucose and lipids (Knapp et al., 2013). Baseline glucose can also vary across populations (e.g. garter snakes) even without tourist exposure (Gangloff et al., 2017); however, differences in diet or energy resources are likely contributing to this variation.

For example, glucose levels were related to food availability across populations of snakes and lizards in the California Channel Islands (Sparkman et al., 2018) and across reproductive stages in northern water snakes (*Nerodia sipedon*) (Kelley et al., 2021). We found significant baseline differences in glucose and CORT according to tourism exposure, whereby populations located adjacent to one another were more different than sites at opposite ends of the Exuma island chain but with similar anthropogenic exposure.

The effects of tourism exposure on CORT, however, are more complex. For example, Romero and Wikelski (2002) found no difference in baseline CORT related to tourism in Galapagos marine iguanas (*Amblyrhynchus cristatus*). However, French et al. (2017) found that CORT varied significantly with tourism in reproductive Galapagos marine iguanas on a different island, whereby the direction of the relationship varied based on sex (male CORT levels increased and female levels decreased with increasing tourism intensity). Moreover, one effect of CORT is to mobilize energy by regulating long-term energy availability via gluconeogenesis in the liver and altering tissue uptake of glucose in the periphery (Kuo et al., 2015; Munck and Koritz, 1962; Romero and Beattie, 2022). Thus, long-term alteration of energy levels (e.g. glucose, fructose) resulting from supplemental feeding may be related to basal CORT. For instance, CORT was elevated in fasted common water snakes (*Nerodia sipedon*) relative to recently fed individuals (Webb et al., 2017). Therefore, in the current study, CORT may be lower in rock iguanas experiencing heavy tourist feeding simply because energy resources are in high abundance. It is also likely that central nervous activation comes into play; however, we were unfortunately unable to measure catecholamines in the current study.

Response to a glucose challenge in free-ranging rock iguanas

Following a glucose challenge, tourist-fed iguanas showed a more a rapid increase, greater peak and slower decrease in blood glucose relative to conspecifics not visited by tourists. Natural variations in glucose response to controlled stressors have been reported in other species in the absence of tourist feeding (Neuman-Lee et al., 2020). For example, Kelley et al. (2021) found stress-induced increases in glucose that varied across reproductive stages in northern water snake (*Nerodia sipedon*) but not for the pygmy rattlesnake (*Sistrurus miliarius*). In gartersnakes, glucose increased in response to a controlled restraint stress, and this response varied across populations, but was more similar among populations in closer geographic proximity (Gangloff et al., 2017; Neuman-Lee et al., 2020). However, in the current study, we saw more similar glucose responses among populations exposed to tourism, relative to populations that are closer geographically but unvisited by tourists (e.g. ∼80 km distance relative to ∼1–1.8 km distance). It is not yet clear whether the differences in glucose levels across populations of reptiles may have resulted from potentially adaptive differences in acute stress responses to common environmental pressures, either geographically (Kelley et al., 2021) or tourism related (this study).

Comparing across taxa, we found relatively higher glucose levels in the iguanas in our study relative to snake species (Telemeco et al., 2019). Other studies in lizards also reported higher glucose levels than in snakes, but not necessarily stress-induced increases in glucose (Dunlap and Schall, 1995; Hudson et al., 2020). In fact, studies in plateau side-blotched lizards (*Uta stansburiana uniformis*) and eastern fence lizards (*Sceloporus occidentalis*) actually find significant decreases in glucose following a stress challenge (Dunlap and Schall, 1995; Hudson et al., 2020).

In contrast to these previous studies that performed controlled stressors and looked at glucose responses, we provided supplemental glucose for our iguanas to metabolize following baseline sampling. The glucose response in rock iguanas was much greater than those previously reported in the absence of a glucose challenge in other reptiles (i.e. orders of magnitude higher), and was not related to body condition, which did not significantly vary among groups. It is possible that the iguanas also increased blood glucose in response to experimental handling (e.g. stress) and not only due to the glucose challenge, but this response would conflict with many other lizard glucose studies to date showing the opposite (Dunlap and Schall, 1995; Hudson et al., 2020). Therefore, it is plausible that if field logistics had allowed for non-glucose control treatments we would have observed similar patterns to those in glucose-treated animals, but with considerably lower overall blood glucose concentrations, as found in previous studies that included a control in birds and snakes (Montoya et al., 2020; Prado, 1946). Regardless, we found significantly higher glucose peaks in tourist animals from geographically distant populations relative to their adjacent islands that are unvisited by tourists. Moreover, we saw a similar response in the laboratory study (study 1), whereby animals provided with high levels of glucose for 17 days had significantly higher peaks in blood glucose following a glucose tolerance test than did control animals. Together, these results show that glucose treatment effectively elevated blood glucose and that iguanas with increased dietary intake of glucose differed significantly in their ability to metabolize the extra glucose provided.

Changes in glucose metabolism akin to what we have observed in the present study are the hallmark of glucose intolerance in better studied species (i.e. humans, mice, rats; Surwit et al., 1988; Abdul-Ghani and DeFronzo, 2009; Surwit et al., 1988). There is some evidence that glucose regulation in reptiles may be similar to that in humans (Penhos and Ramey, 2015). For example, Coulson and Hernandez (1953) performed glucose tolerance tests in American alligators (*Alligator mississippiensis*) and showed that insulin treatment blunted blood glucose peaks, suggesting insulin helps animals clear glucose from the blood. Moreover, spiny-tailed lizards (*Uromastyx aegyptia*) that had undergone a pancreatectomy showed significantly elevated glucose levels above those with an intact pancreas and were described as having a ‘diabetic-like response’, suggesting that insulin is important in regulating blood glucose in reptiles (Khalil and Yanni, 1959). Importantly, as in mammals, glucagon also seems to have a significant role in regulating glucose in reptiles (Penhos and Ramey, 2015). Two studies have shown that long-term glucagon treatment resulted in hyperglycemia (Marques, 1967; Putti et al., 1986).

In addition to changes in blood glucose, we likewise saw greater increases in CORT in tourist-fed animals during glucose tolerance tests. This is similar to the elevated post-stress levels of CORT observed in non-breeding marine iguanas exposed to tourism (French et al., 2010), although a different study in marine iguanas found the opposite response (Romero and Wikelski, 2002). Interestingly, the CORT levels in fed animals 1 h post glucose challenge were positively related to blood glucose levels, suggesting they may be linked. Similarly, glucose was positively related to CORT following a controlled stress response in Florida cottonmouth snakes (*Agkistrodon conanti*), and the response was greater in the population with presumably greater food availability (Sandfoss et al., 2020). This association has also been experimentally documented in alligators treated with cortisone, whereby they responded with significant increases in blood glucose (Coulson and Hernandez, 1953). However, even though glucose was elevated following an acute stressor in both wandering garter snakes (*Thamnophis elegans vagrans*) and common garter snakes (*Thamnophis sirtalis*), no direct relationship was found between stress-induced increases in CORT and blood glucose (Gangloff et al., 2017; Neuman-Lee et al., 2020), suggesting that CORT is not directly mediating glucose changes in the blood. Likewise, there was no relationship at 3 h after glucose challenge or in iguanas on non-visited islands in our study. Recent research suggests that glucocorticoids primarily act to reduce tissue uptake of glucose rather than release, thereby preserving glucose for recovery from a stressor after faster-acting catecholamines induce energy costs (Romero and Beattie, 2022). This mechanism may help explain the lack of a direct relationship between stress-induced CORT and glucose; however, the timeframe for this proposed mechanism does not align with that of the current study. Regardless, glucose tolerance testing along with plasma glucocorticoid measurements may serve as important sentinel metrics to assess the effects of dietary changes on glucose regulation in wild animals.

Finally, it is also possible that supplemental feeding is altering overall dietary choice in iguanas. For example, humans on high sugar diets become dependent on consuming high sugar foods (Avena, 2007). As natural fruits, albeit less sweet than grapes, are at times present on the islands, future work should further investigate the diet composition of grape-fed and non-fed iguanas to determine whether additional dietary changes are occurring in grape-fed animals (e.g. choosing different foods), thus compounding the tourist feeding effects.

Conclusions

We found significant physiological changes in glucose metabolism in response to tourism-associated and lab-based supplemental feeding of sugar-rich dietary components, ultimately resulting in chronic elevations in blood glucose and an altered glucose tolerance response. Moving forward, it will be critical to explore the mechanisms underlying these changes and whether they impact general health and survival. For example, while we have not measured blood glucose regulatory hormones (i.e. insulin and glucagon) directly as of yet, it is an important next step, because there are significant and specific indicators of altered glucose regulation in both laboratory-housed and free-living iguanas. Moreover, other dietary factors in unnatural diets at tourist sites could be compounding the tourism effects as is the case with lipids in humans (Boden and Laakso, 2004; Johnson et al., 2013). In humans, long-term dysregulation in glucose results in significant health consequences and even death. Therefore, understanding the long-term health ramifications of unnaturally high sugar diets will be important both for ensuring sustainable tourism and guaranteeing the conservation of this critically endangered Bahamian iguana species. Given that glucose regulation seems highly conserved across vertebrates, the implications of this research are important for other species experiencing diet alterations.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.S.F., D.F.D.; Methodology: S.S.F., D.F.D.; Validation: D.F.D.; Formal analysis: S.S.F., S.B.H.; Investigation: S.S.F., A.C.W., C.R.K., E.E.V., G.D.S., E.L.L., J.B.I., D.F.D.; Resources: S.S.F., C.R.K., J.B.I.; Data curation: S.S.F., C.R.K., J.B.I., D.F.D.; Writing - original draft: S.S.F., S.B.H., D.F.D.; Writing - review & editing: S.S.F., S.B.H., A.C.W., C.R.K., E.E.V., G.D.S., E.L.L., J.B.I., D.F.D.; Visualization: S.B.H., S.S.F.; Supervision: S.S.F.; Project administration: S.S.F., D.F.D., C.R.K.; Funding acquisition: S.S.F., C.R.K., J.B.I., D.F.D.

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Data availability

Data are freely accessible from USU Digital Commons at: https://doi.org/10.26078/94W0-3V59.

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