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Seasonal and elevational variation in glucose and glycogen in two songbird species

Karen L. Sweazea[a,b,*], Krystal S. Tsosie[b], Elizabeth J. Beckman[c], Phred M. Benham[c], Christopher C. Witt[d]

[a] College of Health Solutions, Arizona State University, United States of America
[b] School of Life Sciences, Arizona State University, United States of America
[c] Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, United States of America
[d] Department of Biology and Museum of Southwestern Biology, University of New Mexico, United States of America

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ABSTRACT

Birds naturally maintain high glucose concentrations in the blood and tissues, even when relying on fat to meet the metabolic demands of flight or thermogenesis. One possibility is that high glucose levels might be needed to deal with these metabolic demands. Thus, we hypothesized that birds chronically exposed to colder temperatures and higher elevations have higher circulating glucose and tissue free glucose and glycogen compared to conspecifics living at warmer temperatures and lower elevations. Adult House Sparrows (*Passer domesticus*) and House Finches (*Haemorhous mexicanus*) were captured from Phoenix, AZ (340 m elevation), and Albuquerque, NM (1600 m elevation), during the summer and winter months. We measured plasma glucose, as well as free glucose and glycogen from multiple tissues. In general, high elevation and colder temperatures were associated with higher tissue glycogen and higher free glucose concentrations in the brain. These findings indicate that glucose and glycogen are subject to seasonal phenotypic flexibility as well as geographic variations that may relate to local food availability and abundance.

1. Introduction

Birds generally have 1.5–2.0-fold higher circulating glucose concentrations than mammals of similar body mass (Beuchat and Chong, 1998; Braun and Sweazea, 2008). The functional explanation for this difference is unclear, but it has been proposed that carbohydrate stores in avian muscle and liver function to provide a quick energy source for take-off and short flights (George and Berger, 1966; Rothe et al., 1987). Another factor that might contribute to high plasma glucose concentrations is that birds are resistant to the glucose-lowering effects of insulin, such that very high concentrations are required to elicit a hypoglycemic effect (Chida et al., 2000; Hazelwood and Lorenz, 1959; Sweazea and Braun, 2005).

When the supply of glucose from the diet or gluconeogenesis exceeds the nutritional needs of an animal, excess is stored mainly in liver or muscle tissues as glycogen, a polymer of glucose. Increased synthesis also occurs after exercise to replenish glycogen stores. Other tissues that can produce glycogen include the kidney, adipose, brain and heart (Roach et al., 2012). Free glucose concentrations within tissues may thus reflect an increase in uptake for immediate metabolic needs or storage for later use. Since muscle lacks glucose-6-phosphatase, this store is only available for use as a glycolytic fuel within muscle. In contrast, since the liver consumes mostly fatty acids for metabolism, the glycogen stored within this tissue provides a readily available source of energy for the body (Hers, 1976). The importance of glycogen reserves to the avian nervous system is evidenced by the observation that birds are the only vertebrate known to have a glycogen body within the spinal cord (Möller and Kummer, 2003) that may act as a reserve to ensure a constant supply of glucose to the nervous system.

High plasma-glucose in birds is curious as studies have shown species rely on free fatty acids (FFA) to fuel long distance flight (Blem, 1990; Guglielmo, 2018; Jenni-Eiermann et al., 2002; Landys et al., 2005). While the ultimate reasons birds maintain high plasma glucose are not known, and are beyond the scope of the current study, it is important to understand how seasonal and geographic variation in glucose and glycogen storage may be associated with variables such as temperature and elevation. For homeotherms, cold is linked with a suite of stressful stimuli, including reduced food availability, shorter days, and higher energy demands for thermoregulation (Collins, 1989). It has been shown in mammals that carbohydrate metabolism increases with

* Corresponding author at: Arizona State University, 401 E Tyler Mall, Mail Code 4501, Tempe, AZ 85287-4501, United States of America.
E-mail address: Karen.Sweazea@asu.edu (K.L. Sweazea).

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Table 1
Average summer and winter temperatures (degrees Fahrenheit ± SEM) at each elevation from which birds were captured.

	Summer			Winter		
	Low	High	Mean	Low	High	Mean
Phoenix, AZ (340 m)	84.0 ± 4.0	107.9 ± 3.6	95.8 ± 3.6	46.5 ± 0.87	71.6 ± 2.2	58.4 ± 1.0
Albuquerque, NM (1600 m)	55.9	88.0	71.2	34.8 ± 1.9	59.0 ± 7.5	47.6 ± 5.6

Mean values were not applicable to birds captured in Albuquerque during the summer as all animals were collected on the same day, September 28, 2011.

cold stress (Chaffee and Roberts, 1971) and altitude (Lau et al., 2017; Schippers et al., 2012). Fuel selection in mammals shifts towards carbohydrates with increasing exercise intensity as long as sufficient carbohydrates are available (from glycogen stores and/or dietary intake), and specifically as they approach the upper extent of their aerobic scope (Edwards, 1934; Schippers et al., 2014). Recent studies show birds shift towards fatty acid metabolism in response to increases in metabolic demands induced by cold stress (Stager et al., 2015; Zhang et al., 2015), high altitude (Qu et al., 2013) as well as long distance flight (Guglielmo, 2018). Although, Dawson et al. (1992) report that glycogen may play a minor role in cold-induced thermogenesis. Studies of maximal oxygen uptake (VO2max) have shown that birds at the upper limit of their aerobic scope, i.e. flying, utilize predominantly fatty acids (respiratory quotient, RQ = 0.73 for pigeons in a wind tunnel (Rothe et al., 1987), which is similar to measured VO2 max of several species while fasting at rest; RQ = 0.71 for house sparrows (Walsberg and Wolf, 1995), 0.72 for hummingbirds (Suarez et al., 1990), and 0.80 for ravens (Hudson and Bernstein, 1983). Moreover, isolated pectoralis muscle mitochondria from house sparrows selectively utilize fatty acids when given a choice between carbohydrates and fatty acids for fuel (Kuzmiak-Glancy and Willis, 2014). This is not the case for all birds, however, as hummingbirds appear to rely primarily on glucose and perhaps fructose for metabolic needs during hovering flight (Welch Jr. and Suarez, 2007). Considering all of this evidence together, it is unlikely that metabolic demands of routine flight alone are a general explanation for high plasma-glucose concentrations in birds. Moreover, studies have demonstrated systematic shifts from carbohydrate to FFA use in birds exposed to decreases in environmental temperature (Stager et al., 2015; Zhang et al., 2015).

We hypothesized that birds living under colder temperatures and/or at higher elevations will have more circulating glucose and tissue concentrations of free glucose and glycogen compared to conspecifics living under warmer temperatures and/or at lower elevations. We chose to include House Sparrows (*Passer domesticus*), an urban exploiter, as well as House Finches (*Haemorhous mexicanus*), an urban adapter, in our study as these species differ in their reliance on human-derived nutrients. While both species consume seeds from backyard feeders, House Sparrows also consume human refuse (breads, chips, etc), which can impact their nutritional physiology (Gavett and Wakeley, 1986). In fact, a prior study showed that urban house sparrows had higher plasma glucose concentrations and a pro-inflammatory gut microbiome than rural counterparts (Gadau et al., 2019). Thus, we may predict that house sparrows may have higher circulating glucose concentrations compared to finches as well.

2. Methods

2.1. Animals

Adult House Sparrows (*Passer domesticus*) and House Finches (*Haemorhous mexicanus*) of both sexes were collected in the morning under fed conditions during the summer and winter months of 2011–2012 in Phoenix, Arizona (~340 m above sea level; atmospheric pressure ~ 730 mmHg; PO_2 ~ 153 mmHg) and Albuquerque, New Mexico (~1600 m above sea level; atmospheric pressure ~ 620 mmHg; PO_2 ~ 130 mmHg), using mist nets. Sample sizes for Phoenix included 6 finches and 5 sparrows during the summer as well as 6 finches and 7 sparrows during the winter. Sample sizes for Albuquerque included 4 finches and 4 sparrows captured during the summer as well as 6 finches and 6 sparrows captured during the winter. Average temperatures are higher in Phoenix than Albuquerque year-round, including during the collection periods (Table 1). Blood samples were collected immediately after capture for the analysis of plasma glucose. Animals were humanely killed by carbon dioxide asphyxiation or rapid cardiac compression and tissues (cardiac and pectoralis muscles, liver, and cerebrum) were collected and frozen at −80 °C until glycogen and free glucose concentrations were measured as described below. All animal protocols were approved by the Arizona State University and University of New Mexico Institutional Animal Care and Use Committees and were collected under appropriate federal and state scientific collecting permits.

2.2. Measurement of plasma and tissue free glucose

Plasma glucose was quantified using a colorimetric assay kit (10,009,582, Cayman Chemical, Ann Arbor, MI, USA). Free glucose concentrations in tissues were measured as previously described (Feillet et al., 2006). Briefly, 50 mg of tissue was lysed in 75 μL of 1 M potassium hydroxide at 95 °C then neutralized with 75 μL of 1 M hydrochloric acid. The resulting homogenate was centrifuged at 13,000 rpm for 10 min at room temperature and free glucose concentrations were assayed in duplicate on a 5 μL aliquot of the supernatant using a colorimetric assay kit according to the manufacturer's protocol (Cayman Chemical, Ann Arbor, MI, USA).

2.3. Measurement of tissue glycogen

Tissue glycogen concentrations were assayed using the phenol-sulfuric acid technique (Lo et al., 1970). Briefly, 50 mg of frozen tissue with all visible fat and connective tissue removed was placed into a polypropylene test tube containing 500 μL of 30% potassium hydroxide solution saturated with sodium sulfate and boiled for 20 min until digested. Glycogen was precipitated by adding 550 μL of 95% ethanol, incubating on ice for 30 min, followed by centrifugation at 800 *g* for 30 min. The resulting supernatant was discarded and the glycogen precipitate was dissolved in 3 mL deionized water. A 1 mL aliquot of the re-suspended glycogen pellet was transferred to a fresh polypropylene test tube for the colorimetric reaction that was initiated by the addition of 1 mL of 5% phenol followed by 5 mL of 96–98% sulfuric acid. After standing for 10 min, the mixture was incubated at 25–30 °C for 15 min prior to measuring the absorbance at 490 nm (iMark plate reader, BioRad, Hercules, CA, USA). A standard curve was created on each plate using glycogen from bovine liver, type IX (Cat. G0885; Sigma Aldrich, St. Louis, MO, USA). All samples were assayed in duplicate. Glycogen content was determined by the following equation (Lo et al., 1970): grams of glycogen/100 g tissue = (A490/k) x (V/v) x (10^{-4}/W). Where k = slope of the standard curve; V = total volume of glycogen solution (in mL); v = volume of aliquot to which phenol-sulfuric acid solution is added (in mL); A_{490} = absorbance at 490 nm; W = sample

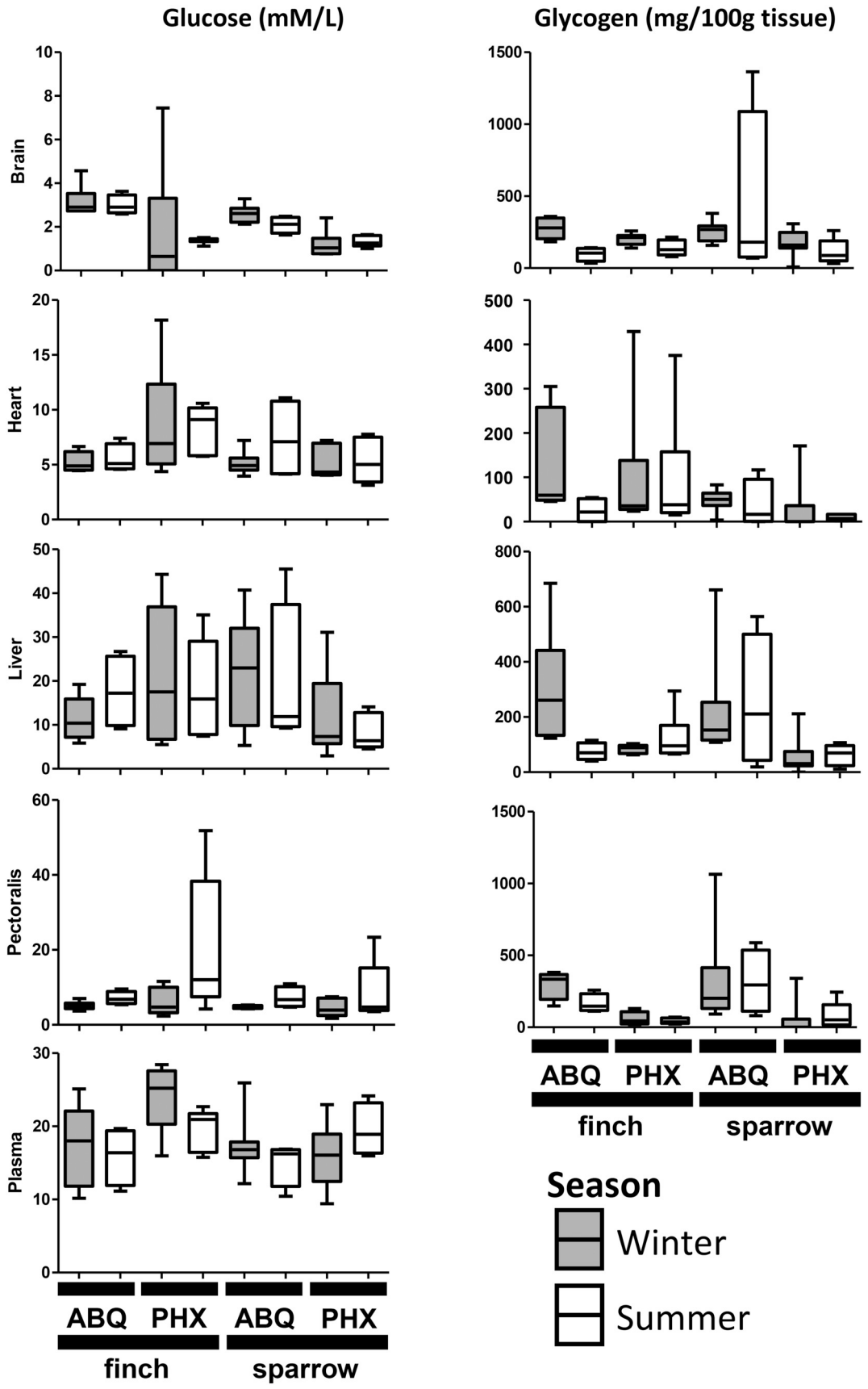

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Fig. 1. Glucose (left) and glycogen (right) concentrations for House Finches and Sparrows. Data analyzed by three-way ANOVA with Student-Newman-Kuels posthoc analyses. Data expressed as mean ± SEM. Posthoc analyses reveal significant effects of elevation within finches on cardiac glucose concentrations ($p = .008$) and within sparrows on liver free glucose concentrations ($p = .038$). Additional significant effects of species were observed within Phoenix on cardiac ($p = .004$) as well as liver free glucose concentrations ($p = .050$). Phoenix sample sizes: summer finches $n = 6$ ($n = 5$ pectoralis data); winter finches n = 6; summer sparrows n = 5 ($n = 4$ plasma glucose); winter sparrows $n = 7$. Albuquerque sample sizes: summer finches n = 4; winter finches n = 6 (n = 5 plasma glucose); summer sparrows n = 4; winter sparrows n = 6 tissue glucose, n = 7 plasma glucose and heart glycogen, $n = 8$ liver, pectoralis and brain glycogen concentrations. Variations between animal numbers and tissue sample sizes reflect size of available tissue for analyses.

Table 2
Summary of three-way ANOVA analyses of data presented in Fig. 1.

Variable	Species (finch vs sparrow)	Elevation (ABQ vs PHX)	Season (Winter vs Summer)	Species* Elevation	Species* Season	Elev. * Season	Species * elev. * Season
Brain Glucose (P_{GLU})							
F	2.123	12.926	0.437	0.389	0.017	0.078	0.370
P-value	0.154	<**0.001***	0.513	0.537	0.898	0.782	0.547
Heart Glucose							
F	2.416	1.813	0.635	6.834	0.607	0.623	0.181
P-value	0.129	0.187	0.431	**0.013***	0.441	0.435	0.673
Liver Glucose							
F	0.205	0.640	0.031	5.122	0.420	0.497	0.336
P-value	0.653	0.429	0.862	**0.030***	0.521	0.485	0.566
Pectoralis Glucose							
F	2.445	2.924	6.696	2.301	1.231	2.516	1.465
P-value	0.127	0.096	**0.014***	0.138	0.275	0.122	0.234
Plasma Glucose							
F	3.047	6.565	0.650	2.245	1.658	0.399	3.128
P-value	0.090	**0.015***	0.425	0.143	0.206	0.532	0.086
Brain Glycogen							
F	1.486	4.030	0.227	3.011	2.819	0.346	2.643
P-value	0.230	0.052	0.637	0.091	0.101	0.560	0.112
Heart Glycogen							
F	3.695	0.001	1.402	0.604	0.417	0.582	0.891
P-value	0.062	0.974	0.244	0.442	0.523	0.452	0.351
Liver Glycogen							
F	0.001	10.046	0.921	1.254	1.806	2.223	3.199
P-value	0.971	**0.003***	0.343	0.270	0.187	0.144	0.082
Pectoralis Glycogen							
F	1.049	17.008	0.420	0.490	0.563	0.497	0.149
P-value	0.312	<**0.001***	0.521	0.488	0.458	0.485	0.702

Statistical analyses of data presented in Fig. 1. n = 4–8 birds per group.

weight (g). All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

Statistical Analyses: Three way-ANOVA was used to test for variations in glucose and glycogen concentrations with species (finch vs sparrow), elevation (Phoenix vs Albuquerque) and season (summer vs winter) as factors. All statistical analyses were completed using SigmaPlot 14.0 Software (Systat Software Inc. Chicago, IL, USA). Where significant effects were observed, Student-Newman-Keuls posthoc analyses were used. Spearman rank correlation (SPSS Statistics Version 21, IBM) was used to test for relationships between plasma glucose and liver glycogen concentrations for each species. For the purpose of interpreting our results, 'elevation' can be equated with 'population site' because we sampled from only two sites that differ in elevation. All data are provided in Supplemental Table 1. For all comparisons, $p \leq .05$ was considered statistically significant.

3. Results

Fig. 1 shows plasma glucose as well as tissue glucose and glycogen concentrations. Results from three-way ANOVAs of data in Fig. 1 revealed no significant effects of species on glucose or glycogen concentrations for any variable examined (Table 2). When data are combined for species, Albuquerque birds had higher brain free-glucose but lower plasma glucose than birds captured in Phoenix (Table 2). Similarly, Albuquerque birds had higher liver and pectoralis glycogen concentrations (Table 2). Pectoralis muscle free glucose concentrations were higher in birds during the summer as compared to winter. (See Table 2.)

Secondary post-hoc analyses revealed significant differences between and within species for heart and liver glucose concentrations. Finches captured from Phoenix had significantly higher free glucose concentrations in the heart than both finches captured from Albuquerque ($p = .008$) and sparrows captured from Phoenix ($p = .004$). Additionally, sparrows captured in Albuquerque had significantly greater liver free glucose concentrations than sparrows captured in Phoenix ($p = .038$) although finches captured in Phoenix maintained higher liver free glucose concentrations compared to sparrows ($p = .050$).

Liver glycogen was negatively correlated with plasma glucose in House Sparrows (Fig. 2,Table 3), but no other significant relationship was observed between plasma glucose and tissue glucose or glycogen.

4. Discussion

We report tissue free-glucose and glycogen concentrations varied predictably with season and elevation. In general, the data supported the hypothesis that higher elevations and colder temperatures were associated with increased concentrations of free glucose and glycogen within tissues. However, the finding that plasma glucose was lower at high elevations and colder temperatures does not support our hypothesis. Moreover, sparrows did not have higher circulating glucose concentrations than finches.

Glucose is the major fuel substrate used by the brain. In fact, prior studies of Mourning Doves (*Zenaida macroura*) captured from low elevations have shown that the brain takes up significantly more glucose in comparison to skeletal muscle and liver (Sweazea et al., 2006).

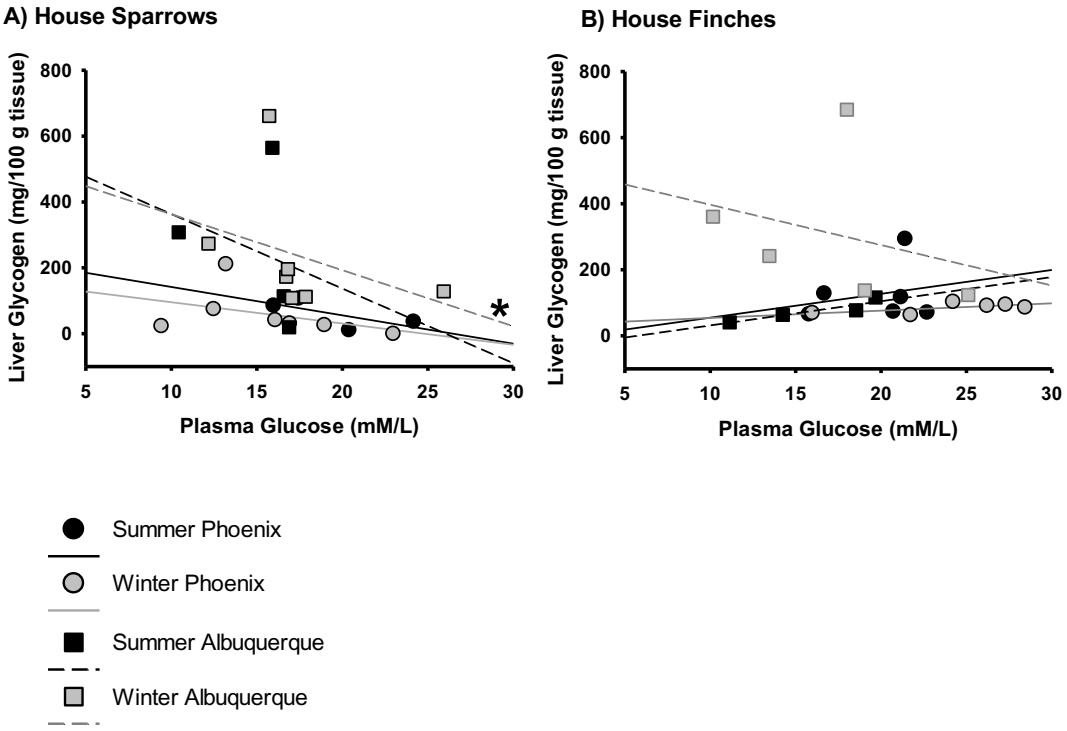

Fig. 2. Correlations between plasma glucose and liver glycogen for house sparrows (left) and house finches (right). Phoenix sample sizes: summer finches n = 6; winter finches n = 6; summer sparrows n = 4; winter sparrows n = 7. Albuquerque sample sizes: summer finches n = 4; winter finches n = 6; summer sparrows n = 4; winter sparrows *n* = 8. Variations between animal numbers and tissue sample sizes reflect size of available tissue for analyses. Symbols on graphs represent individual birds. Lines depict regression analyses. Spearman correlations showed a significant negative correlation between plasma glucose and liver glycogen concentrations for sparrows captured during the winter in Albuquerque (Spearman correlation coefficient (ρ) = −0.786, **p*-value = .0251). No other significant relationships were observed.

Table 3
Correlation coefficients for tissue metabolites and plasma glucose in free-living birds.

	House Sparrows	House Finches	All Birds
	P_{glu}	P_{glu}	P_{glu}
Heart Free Glucose	0.338	−0.197	0.150
Heart Glycogen	−0.131	0.177	0.187
Liver Free Glucose	0.119	−0.170	0.014
Liver Glycogen	−0.459*	−0.009	−0.185
Pectoralis Free Glucose	−0.204	−0.209	−0.143
Pectoralis Glycogen	−0.165	−0.332	−0.197
Brain Free Glucose	−0.077	−0.292	−0.135
Brain Glycogen	−0.076	0.021	−0.038

Spearman order correlations**p* < *.05*.

Likewise, data from the current study show that birds living at high elevation have higher free glucose concentrations in the brain but lower plasma glucose concentrations. The high concentration of free glucose in the brain may be attributed to higher uptake from the blood, which could explain the lower concentrations of circulating glucose in these birds. Although variations in food availability and abundance between sites may also explain the lower plasma glucose concentrations at higher elevation.

The birds examined in the present study also had high concentrations of glycogen in the brain relative to many other tissues. Although concentrations of glycogen within the brains of mammals are an order of magnitude lower than in their muscles, they are still higher than those of free glucose (Gruetter, 2003), making glycogen the major energy reserve for the brain (Cruz and Dienel, 2002). Further, studies in mammals have shown that glial glycogen can supply neurons with glucose for up to 100 min during hypoglycemia (Gruetter, 2003). Previous studies have suggested that glycogen becomes important in mammals exposed to mild hypoxia (Gruetter, 2003), consistent with higher brain glycogen content in sparrows and finches living at higher elevations. Turtles (*Chrysemys dorbigni*) also exhibit higher brain glycogen content during winter, when they experience hypoxia (Partata and Marques, 1994).

We found that birds at the higher elevation, colder site had higher glycogen concentrations within the liver and pectoralis muscle. These findings are consistent with previous findings for American goldfinches (*Spinus tristis*) showing higher flight-muscle glycogen content during winter compared to summer, a result that was attributed to the need to utilize glucose for the high metabolic demands of cold stress (Carey et al., 1978). However, recent data suggest that birds may shift towards FFA metabolism for this purpose (Stager et al., 2015; Zhang et al., 2015). Since Albuquerque is colder than Phoenix at all seasons, we have limited power to distinguish temperature effects from elevation effects in this study. A likely explanation for the higher tissue glycogen concentrations during cold is that levels rise to meet the higher metabolic demands during cold stress (Carey et al., 1978).

In contrast, plasma glucose levels were higher in birds captured from the lower elevation, warmer site. These findings are consistent with the results of a study on American goldfinches (*S. tristis*) examined during the summer and winter (Marsh et al., 1984). In that study, the authors concluded that glucose metabolism did not vary with exposure to cold and that goldfinches rely more on FFA oxidation during cold weather (Marsh et al., 1984). While a shift towards carbohydrate use with an increase in elevation has been documented for Andean rodents (Lau et al., 2017; Schippers et al., 2012), recent studies demonstrate a shift towards FFA metabolism in birds living at high elevations (Qu et al., 2013). This shift towards FFA metabolism may also explain the

higher storage of glucose as glycogen in birds captured from the colder site as less glucose may be consumed for metabolism. A recent study comparing transcriptomic profiles in the pectoralis muscles of American goldfinches (*S. tristis*) and black-capped chickadees (*Poecile atricapillus*) showed more than 1200 genes were altered with seasonal changes. This corresponded with changes in metabolic and regulatory pathways (Cheviron and Swanson, 2017). Thus, it is likely that the birds in the present study have similar phenotypic flexibility that allows them to adapt to changing environments and seasons.

In conclusion, the results from this study revealed variations in tissue free glucose and glycogen concentrations for two species of birds in a high altitude and moderately cold environment compared to conspecifics residing in a low altitude warmer environment. Glycogen is primarily used for burst flight activity (Guglielmo, 2018). Birds may require more glycogen at high altitude to overcome the challenge of decreased air density for take-off (Scott, 2011). However, glycogen is also a fuel for non-shivering thermogenesis in birds (George, 1982). The seasonal variation that was evident in both glucose and glycogen levels may also suggest a role for seasonal phenotypic flexibility in the regulation of glucose and glycogen concentrations, but we cannot rule out that genetic local adaptation may have also contributed to the variation that we observed between populations separated by ~1260 m. As birds were not fasted prior to blood collection, it was not possible to assess whether variations in blood glucose are related to differences in the availability of foods between seasons and locations. Moreover, birds were randomly sampled from the environment, therefore diet composition was not measured. Thus, it was not possible to determine whether variations in diet contributed to these observed differences. Combining comparative and experimental approaches will be key to better understand the relative importance of different fuel sources for different activities (e.g. flight vs. thermoregulation), ambient climatic conditions, and seasons.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2020.110703.

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