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Glycated hemoglobin and albumin reflect nestling growth and condition in American kestrels

Daniel R. Ardia *

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, 18453, USA

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Abstract

Blood chemistry can be used to assess physiological state and condition. Levels of glycated hemoglobin (GHb), which integrates blood glucose levels over a period of weeks, may provide a way to assess resource intake. I tested whether GHb reflects offspring quality by comparing growth rates of nestling American kestrels (*Falco sparverius*) with GHb levels at 24 days of age. Nestlings that gained structural size faster had higher levels of GHb than did slower growing nestlings. There was no difference in GHb levels between males and females, although females are larger. In addition, I tested whether albumin levels, a measure of protein storage, were correlated with nestling growth and body condition (reflected in residual body mass). Larger individuals, measured by both absolute body mass and by residual body mass, had larger levels of albumin. This was due in part to females having higher albumin levels. Interestingly, there was no correlation between GHb and albumin, suggesting that both measures are necessary to assess physical condition in nestling kestrels. These results suggest that blood chemistry can reflect offspring condition measures and may provide a way to assess offspring quality that reflects conditions experienced by offspring over longer periods through measurements at a single time point.

Keywords: Glycated hemoglobin; Plasma protein; Blood chemistry; Offspring quality; Growth rate; Body condition; *Falco*

1. Introduction

Blood chemistry can reveal physiological state in free-living organisms, often by reflecting levels of nutrient stores, immune status, or stress levels, and is frequently used to assess condition (Dawson and Bortolotti, 1997; Rea et al., 1998; van Wyk et al., 1998; Totzke et al., 1999; Grasman et al., 2000). The use of physiological measures to assess offspring quality in birds is particularly important because nestling birds return at low rates, thus making it difficult to follow individuals over time (Perrins, 1964; Hochachka and Smith, 1991; Christe et al., 2001). A recent measure of condition described in adult birds, the fraction of hemoglobin that is glycated or glycosylated (GHb) (Andersson and Gustafsson, 1995), has not yet been examined as an indicator of physical condition in nestling birds.

Levels of glycated hemoglobin in blood are relevant for ecological studies because they can reflect blood glucose levels, thus indicating differences in access to resources and nutrient stores (Rendell et al., 1985; Miksik and Hodny, 1992; Beuchat and Chong, 1998). Glycation occurs when the structure of proteins is modified through a non-enzymatic interaction with glucose (Goldstein et al., 1986). Glycation occurs in rough proportion to the concentration of glucose in the blood stream, thus high levels of glucose lead to high levels of glycation (Bunn et al., 1976; Alayash et al., 1988). As hemoglobin cells persist in the blood stream until apoptosis occurs, the proportion of glycated hemoglobin can reveal the levels of glucose in blood over the average life span of red blood cells, which ranges from 3 to 5 weeks in birds (Harrison and Harrison, 1986; Andersson and Gustafsson, 1995). While assessing levels of glycated hemoglobin is a standard practice in human diabetes diagnosis (Gabbay et al., 1977; Bunn et al., 1978; Goldstein et al., 1994), it has only recently been assessed in non-human animals. Andersson and Gustafsson (1995) found that early arrival date in pied flycatchers (*Ficedula hypoleuca*), a predictor of reproductive success, was correlated with higher levels of glycated hemoglobin, suggesting that GHb levels may indicate quality.

* Current address: Program in Organismic and Evolutionary Biology, Morrill Science Center, University of Massachusetts, Amherst, MA 01003, USA. Tel.: +1 413 545 0035; fax: +1 413 545 3243.
E-mail address: ardia@bio.umass.edu.

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Another physiological measure of condition in birds is the level of protein in blood, which provide important transport, immune and energy functions (Hill and Murray, 1987; Lumeij, 1987; Hõrak et al., 2002). Albumin represents the largest single fraction of protein in healthy individuals and serves as the major reservoir of protein and a main contributor of colloidal osmotic pressure and a transport carrier for small molecules such as vitamins, minerals, hormones and fatty acids (Hill et al., 2004).

The main objective of this study was to test whether GHb and albumin levels reflect measures of physical condition in nestling American kestrels (*Falco sparverius*). In bird species where offspring are dependent upon food deliveries for growth, such as kestrels, growth rate is a robust indicator of resource acquisition and is linked to future condition and reproduction (Quinney et al., 1986; Tinbergen and Boerlijst, 1990; Hochachka and Smith, 1991; Starck and Ricklefs, 1998; Naef-Daenzer and Keller, 1999). Accordingly, I tested whether GHb and albumin levels would be correlated with growth rate and predicted that individuals with rapid growth would have higher levels of GHb and albumin in their blood. In addition, I tested whether GHb and albumin were correlated with residual body mass (often called "body condition index"), which reflects resource stores (Conway et al., 1994; Ardia, 2005). Finally, I tested for correlation among blood chemistry values by investigating whether individuals with high GHb levels also had high levels of albumin in blood.

2. Materials and methods

2.1. General field methods

American kestrels (*Falco sparverius*) were studied in 1998 in Berks, Lehigh and Schuylkill counties, southeastern Pennsylvania, USA (centered on latitude 40°55′N, longitude 75°75′W). The 800 km^2 study area comprised a patchwork of rolling hills and farmlands, consisting primarily of cut agricultural land (corn *Zea mays*, soybean *Glycine max*, alfalfa , and pasture) separated by small woodlots and orchards. Each year, between 70 and 100 pairs of American kestrels nest in the area in approximately 200 nest boxes erected for their use (Rohrbaugh and Yahner, 1997).

Nestboxes were visited every 1 to 3 days until clutches were completed, allowing me to quantify clutch initiation date and clutch size. Nests were then revisited every day beginning 2 days before expected hatching date (based on incubation period of 29 days) to determine date of hatching. A total of 70 nestlings from 21 nests were measured at age 4, 8, 12, 16, 20 and 24 days (day of hatching=day 1). During each visit, I measured body mass with an electronic scale (±0.05 g accuracy), and head–bill length, tarsus length and carpometacarpus length with digital calipers (±0.01 mm accuracy). Chicks were marked with colored nail polish to identify individuals.

2.2. Serology

On nestling day 24, I collected whole blood (100–400 μL) from each nestling via the brachial vein and stored the blood in heparinized tubes. After collection, samples were stored in a cooler in the field and then transported immediately to the laboratory and placed in a refrigerator (2–8 °C). All analyses were conducted on the evening of blood collection.

Total glycated hemoglobin (GHb) was assessed in whole blood using Sigma Procedure No. 442 (Sigma-Aldrich, USA), which utilizes an affinity resin in columns to bind glycated hemoglobin and allowing other hemoglobin fractions to wash through. The percentage of hemoglobin that is glycated is determined by comparing the absorbance (at 415 nm) of the wash fraction vs. the elution fraction, which represents the glycated hemoglobin. All absorbances were measured on a spectrophotometer (Spectronic 20 Genesys, Spectronic Instruments, Rochester, NY, USA). A control level of glycated hemoglobin (Sigma) was run simultaneously for each assessment.

In the portion of the blood sample not used to assess GHb levels, plasma was removed following centrifuging each sample at 14,000×*g* for 3 min. Plasma albumin levels were determined using Sigma Procedure No. 631; levels were determined by comparing the absorbance (at 628 nm) of each sample to the absorbance of a Protein Standard Solution (Sigma 540-10).

2.3. Statistical analyses

All response variables were tested for normality using Shapiro–Wilk's W (all variables had W values greater than 0.97 with P values >0.20). Growth rate from day 4 to day 24 was calculated as the growth rate constant K of a logistic growth function (Starck and Ricklefs, 1998), as logistic growth models fit kestrel growth well (Negro et al., 1994; Massemin et al., 2002). Because of co-variation among nestling growth variables, growth rate of tarsus, head–bill and carpometacarpus were combined in a Principal Components Analysis. The first principal component (PRIN 1) had high positive loadings of all three growth rates and explained 89% of the variation, thus PRIN 1 was used the measure of nestling growth. Body mass was regressed against tarsus length on day 24 to obtain residual body mass (i.e. body condition index) ($F_{1,68}=10.1$, $P=0.002$; $R^2=0.45$). Sex differences in size and growth rate were compared using a mixed model analysis of variance (PROC MIXED) (SAS, 1988) with nestbox as a random effect.

Factors affecting the levels of glycated hemoglobin and albumin in each individual were analyzed using a mixed model analysis of variance (PROC MIXED) (SAS, 1988) with nestbox as a random effect (thus avoiding pseudoreplication in comparisons among individuals) and number of broodmates, growth rate (PRIN 1), residual body mass, absolute body mass and nestling sex as fixed effects. Models were also run using the growth rate of each single structural measure as well as without absolute body mass; there was no difference in the magnitude nor significance in the results relative to the full model previously described, so only the results of the full model are reported here. All two-way interaction terms were included initially in each model and then removed sequentially by highest P-value for those interactions with $P>0.20$. No

interaction terms remained in the model; removal of interactions did not change the magnitude or significance of main effects. Random effects were tested by subtracting the −2 log likelihood scores of a model containing the random effect from the −2 log likelihood score of the model minus the effect being tested (Littell et al., 1996). In addition, *t*-tests were used to compare size and growth variables between males and females. All values are reported as means±S.E.M.

3. Results

3.1. Nestling growth and body size

Female nestlings on day 24 maintained larger body mass than did male nestlings (females=129.8±2.39 g, N=42 and males=117.9±2.87 g, N=28; $F_{1,67}$=7.2, P=0.009), but there was no difference in growth rate between males and females when compared by individual measures (slope of growth curves: tarsus, male=0.55±0.04 and female=0.59±0.03, $F_{1,67}$=0.7, P=0.44; head–bill, male=0.43±0.02 and female=0.44±0.03, $F_{1,67}$=0.9, P=0.35; carpometacarpus, male=0.47±0.04 and female=0.49±0.04, $F_{1,66}$=0.51, P=0.48) or as a single principal component (PRIN 1: male=0.03±0.16 and female=−0.13±0.13, $F_{1,67}$=0.6, P=0.43).

3.2. Factors affecting glycated hemoglobin levels

Levels of glycated hemoglobin in nestlings ranged from 0.62% to 2.71% of total hemoglobin (mean=1.25±0.05%, N=70); there was no difference in glycated hemoglobin levels between males and females (males=1.19±0.07, N=28; females=1.28±0.07, N=42; $F_{1,62}$=1.26, P=0.26). Growth rate of nestlings was highly correlated with levels of glycated hemoglobin at 24 days of age ($F_{1,62}$=56.37, P<0.001; β=0.35, R^2=0.49; Fig. 1). However, there was no relationship between glycated hemoglobin and number of broodmates ($F_{1,62}$=0.68, P=0.41), residual body mass ($F_{1,62}$=1.33, P=0.25) or absolute body mass ($F_{1,62}$=1.04, P=0.47). There was a significant random effect of nest on glycated hemoglobin levels, indicating differences among nests in food intake (χ^2_1=6.8, P=0.001).

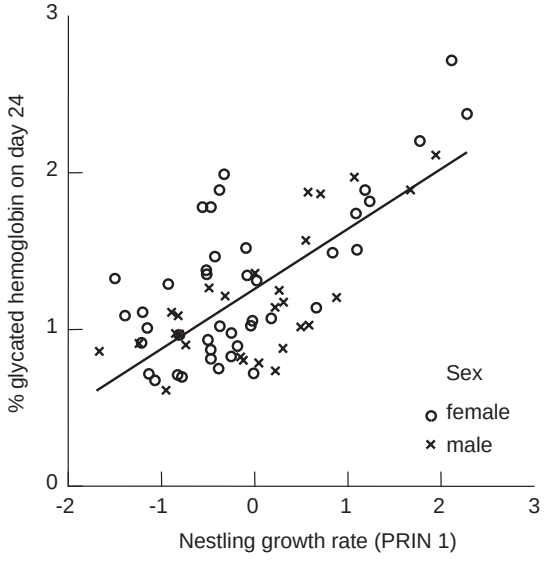

Fig. 1. Correlation between levels of glycated hemoglobin at 24 days of age and growth rate in nestling American kestrels. Growth rate is expressed as the first principal component, which is a composite of rate of growth in tarsus length, head–bill length, and carpometacarpus; higher values represent faster growth.

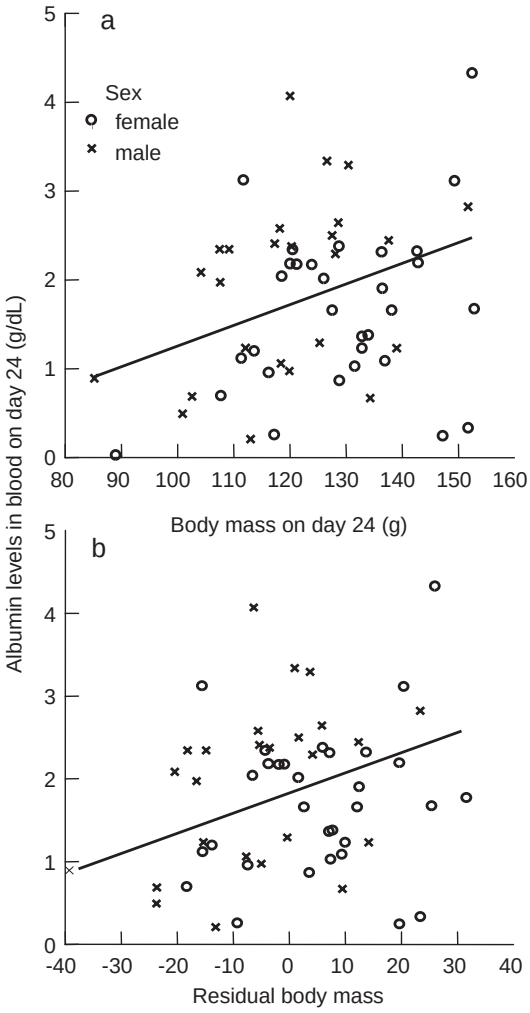

Fig. 2. The relationship between albumin levels at 24 days of age and (a) body mass and (b) residual body mass in nestling American kestrels.

3.3. Factors affecting albumin levels

Albumin levels in chicks ranged from 0.23 to 4.32 g/dl (mean=1.24±0.06 g/dl, N=70); males had higher levels of albumin than did females (males=1.66±0.23 g/dl, N=28 and females=1.22±0.23 g/dl, N=42; $F_{1,62}$=4.07, P=0.04). Larger chicks, controlling for sex, tended to maintain higher levels of albumin ($F_{1,62}$=6.66, P=0.01; Fig. 2a); in addition, nestlings with high residual body mass, regardless of absolute body mass or sex, also tended to have high levels of albumin ($F_{1,62}$=4.23, P=0.04; Fig. 2b). There was no relationship between albumin and the number of brood mates ($F_{1,62}$=0.22, P=0.64) or growth rate ($F_{1,62}$=0.01, P=0.99). Albumin levels were not correlated with levels of glycated hemoglobin ($F_{1,64}$=0.19, P=0.67).

There was a significant random effect of nest on albumin levels ($\chi^2_1 = 4.3$, $P = 0.04$).

4. Discussion

This study tested whether two measures of blood chemistry, glycated hemoglobin (GHb) and albumin, were correlated with growth rate and residual body mass, measures of physical condition, in nestling American kestrels. Nestlings that had rapid structural growth also had higher levels of glycated hemoglobin, while nestlings with high absolute or residual body mass had higher levels of albumin. These results suggest that these measures of blood chemistry may be good predictors of physical conditions, at least in birds, especially glycated hemoglobin which provides an assessment of long-term condition through a single measure.

4.1. Factors affecting variation in GHb

Glycated hemoglobin is believed to integrate levels of blood glucose over an extended period of time and thus should be expected to indicate development conditions over time better than glucose levels in the blood at any single given point in time (Miksik and Hodny, 1992; Rohlfing et al., 2002) or other short-term physiological measures. This indicates that assessing GHb levels, which can be done at a single point in time, may provide information about resource conditions that normally would require multiple visits, such as growth rate. The link between growth rate and GHb levels in this study was tight for a field measure ($R^2 = 0.49$), suggesting that if the relationship is not causal, then factors that affect growth rate have a similar effect on GHb levels. A link between resource intake and GHb is plausible only if nestlings have similar patterns of red blood cell growth and death as do adults, thus making a measure of condition that integrates cellular processes over time appropriate (Gayathri et al., 2004). The levels of GHb in nestlings reported here are similar to levels found in adult kestrels at the same location (unpublished data).

Differences in food intake are believed to cause differences in GHb levels in rats (Gallaher and Schaubert, 1990), deer (Jenks et al., 1991), birds (Miksik and Hodny, 1992), and humans (Rohlfing et al., 2002), although chronic food deprivation does not always reduce GHb levels (Lane et al., 1995). This suggests that factors in addition to resource intake can affect levels of GHb. The next step in assessing the use of GHb would be more closely compare food deliveries, growth and GHb; an attempt was made to record feeding observations in this study, but logistical difficulties reduced sample size.

What other factors than food supply might contribute to differences among individuals in GHb levels? Species-level differences in glycated hemoglobin levels are believed to be caused by differences in erythrocyte membrane permeability to glucose (Higgins et al., 1982) and turnover of red blood cells (Rendell et al., 1985; Rohlfing et al., 2002). However, it is unlikely that individuals of similar age at a single location would differ greatly in cellular level processes; however, without a cross-fostering experiment, it is difficult to separate common genetics from a similar rearing environment.

4.2. Factors affecting albumin levels

Albumin is believed to reflect levels of protein in the diet (Leveille et al., 1962; Gavett and Wakeley, 1986) and thus is generally considered a robust indicator of nutritional condition (Jenni-Eiermann and Jenni, 1998; Hõrak et al., 2002). In a study examining changes in albumin levels over the development period, nestling European starlings (*Sturnus vulgaris*) showed an initial increase in albumin as offspring increased in protein intake and then a plateau, suggesting an individual-level equilibrium between protein intake and usage (Jurani et al., 2004). In this study, albumin levels in blood were not correlated with growth rate, but rather residual body mass, which reflects resource stores, including fat. This suggests that the nutritional intake of fat and protein are linked or that biochemical process controlling fat storage and deposition and protein intake are linked. The lack of a relationship between albumin levels and growth rate is harder to explain, although the lack of linkage suggests that the components of diet that foster rapid growth may be different from those affecting protein retention or that nestlings may make tradeoffs between resource allocation between growth and resource storage. In SE Pennsylvania, kestrels consume a diet of rodents, grasshoppers, snakes and small birds (Ardia, 2002), but more data are needed on differences in diet both among and within nests.

5. Conclusion

One benefit of physiological measures is that they provide an insight into conditions over time through a single time point measure; the results reported here suggest that a single blood collection event can reveal information about glucose and protein intake over the nestling period of American kestrels. However, that there was no correlation between GHb and albumin indicates that each measure provides different but complementary information on the quality of young. Both measures used in concert can reveal important information to assess the complex relationship between offspring quality and development conditions (Hõrak et al., 1998, 2002).

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