SEASONAL VARIATION OF VASCULAR OXYGEN TRANSPORT IN THE DARK-EYED JUNCO[1]

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Abstract. Small birds wintering in temperate climates must increase rates of aerobic metabolism to meet thermoregulatory demands. Increased oxygen delivery to the tissues and/or increased oxygen unloading at the tissues may be involved in meeting this need. This study examines seasonal variation in vascular oxygen transport of Dark-eyed Juncos (*Junco hyemalis*) in western Oregon. Oxygen dissociation curves, hematocrits, and oxygen carrying capacities were determined in summer- and winter-acclimatized juncos. Oxygen affinity did not vary seasonally, P_{50} values for summer and winter were 54.1 torr and 54.0 torr, respectively, at 41°C and pH 7.5. Hematocrit values averaged $47.2 \pm 3.3\%$ in summer and $52.6 \pm 2.0\%$ in winter and were significantly different. Mean values for oxygen carrying capacity, 13.33 ± 1.21 vol % in summer and 14.48 ± 1.20 vol % in winter, also differed significantly. Apparently, increased oxygen demands in winter-acclimatized Dark-eyed Juncos are met, in part, by increased oxygen carrying capacity, but not by decreased oxygen affinity.

Key words: Dark-eyed Junco; Junco hyemalis; oxygen carrying capacity; hematocrit; oxygen dissociation curves.

INTRODUCTION

Small birds wintering in temperate climates show increased cold tolerance during the winter months (Hart 1962, Barnett 1970, Pohl and West 1973, Dawson and Carey 1976, Dawson et al. 1983). However, mechanisms underlying this response are incompletely understood. Apparently, increased cold tolerance in winter-acclimatized small birds depends primarily upon metabolic, rather than insulative, adjustments (Dawson and Carey 1976). Consequently, metabolic rates in winter must be elevated well above basal levels, even at moderately cold temperatures (Dawson and Carey 1976, Dawson et al. 1983). Augmented capacities for aerobic thermogenesis appear necessary to meet winter thermoregulatory demands.

Enhanced shivering thermogenesis appears primarily responsible for increased thermogenic capacity in winter-acclimatized birds (Dawson and Carey 1976). Sustained shivering in response to cold stress results in an increased demand for oxygen in the muscles involved. Therefore, seasonal adjustments in vascular oxygen transport, allowing increased oxygen delivery in winter-acclimatized birds, might be expected. Oxygen unloading to shivering tissues might be increased

by several mechanisms, including increased oxygen carrying capacity, decreased hemoglobin oxygen affinity, increased cardiac output, or altered conditions at the tissues resulting in increased arteriovenous P_{O_2} differences. Shivering efficiency would presumably be enhanced if oxygen delivery could be improved, at low metabolic cost, thereby facilitating increased shivering endurance.

Investigation of possible seasonal variation in avian capacities for vascular oxygen transport has received little attention. Consequently, an overview of avian vascular oxygen transport responses associated with seasonal acclimatization is lacking. Although a winter increment in hematocrit has been reported for some passerines (Carey and Morton 1976, deGraw et al. 1979), seasonal trends in oxygen affinity remain largely uninvestigated. This study examines some aspects of vascular oxygen transport in a small passerine indigenous to western Oregon, the Dark-eyed Junco (Emberizidae: *Junco hyemalis*) to determine if seasonal acclimatization is accompanied by variation in the vascular oxygen transport system.

METHODS AND MATERIALS

Dark-eyed Juncos were captured by mist netting and live trapping at several sites near Corvallis, Benton County, Oregon. Elevation at the capture sites ranged from about 200 m to about 400 m.

After capture, birds were transported to the laboratory, or held overnight in outdoor cages with food and water provided ad libitum. Birds tested from 1 December–28 February 1985–1986, were designated as winter birds, and those tested from 20 May–31 August 1986, as summer birds. All birds were adults. Mean and extreme minimum daily temperatures in Corvallis were 0.4°C and −7.8°C for the winter period and 10.7°C and 3.9°C for the summer period (Oregon State University Climatic Research Institute).

Blood samples were withdrawn from the right jugular vein. Clotting was prevented by rinsing the needle and syringe with 0.05 ml, or less, Na-heparin. No correction was made for this slight change in volume. Since individual samples were small (about 0.5 ml), blood from three to six individuals was pooled for each dissociation curve.

Oxygen dissociation curves (O_2DCs) were generated by a modification of the method of Tucker (1967). The pooled blood sample was placed into a 20-ml water-jacketed (41°C) vessel for equilibration. Equilibration was accomplished by continuously passing water vapor-saturated gas mixtures, containing 5% CO_2 and varying amounts of O_2 and N_2, over the blood, with gentle shaking. Oxygen carrying capacity (O_2Cap) was determined by equilibration with 95% air/5% CO_2 and calculated according to Laver et al. (1965). Use of 95% air/5% CO_2 instead of higher O_2 concentrations probably results in a slight underestimation of O_2Cap and possibly a slight underestimation of P_{50}.

For each O_2DC entry, a 0.1-ml sample was anaerobically withdrawn from the equilibration vessel and placed into a Radiometer Copenhagen BMS 3 Mk 3 Blood Gas Analyzer at 41°C for P_{O_2} and pH determination. A second 0.05-ml sample was anaerobically withdrawn and placed into a 3.0-ml syringe filled with low P_{O_2} potassium-ferricyanide reagent (Tucker 1967). The solution was anaerobically mixed to dissociate all oxygen from hemoglobin and placed into the gas analyzer for P_{O_2} determination. The difference in P_{O_2} between the ferricyanide reagent and the blood/ferricyanide mixture allowed calculation of blood O_2 content and percent saturation (Laver et al. 1965).

A winter O_2DC was generated from nine separate curves composed from the blood of 41 birds, and the summer O_2DC from 11 separate curves from the blood of 45 birds.
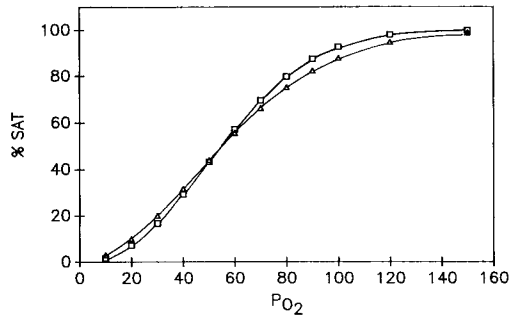

FIGURE 1. Oxygen dissociation curves for summer- (triangle) and winter-acclimatized (square) juncos at 41°C and 5% CO_2 ($P_{CO_2} \sim 38$ torr). Mean values for pH were 7.43 ± 0.04 in summer and 7.46 ± 0.04 in winter. The curves are not significantly different ($P >$ 0.05). The equations are: Winter %Sat = $100(1 - e^{-0.0001[P_{O_2}]^{2.208502}})$ and Summer %Sat = $100(1 - e^{-0.0004[P_{O_2}]^{1.859872}})$.

The Bohr coefficient ($\phi = \Delta\log P_{50}/\Delta pH$) was determined by generation of O_2DCs at varying concentrations of CO_2 (%CO_2 = 0%, 5%, and 12%) in spring and fall. Hematocrit was determined on residual blood from O_2DCs by centrifugation in a microhematocrit centrifuge (International Clinical Centrifuge) for 6 min.

Data on dissociation were fitted by nonlinear regression to a Weibull distribution describing nonsymmetric sigmoid curves, according to the equation:

$$\%Sat = 100(1 - e^{B_0[P_{O_2}]^{B_1}})$$

Weibull parameters, B_0 (scale parameter) and B_1 (curve shape parameter), were evaluated by the t-test. The data for hematocrit and oxygen carrying capacity are presented in terms of $\bar{x} \pm$ SD. Sample comparisons were made using student's t-test. Statistical significance was accepted at the 0.05 level.

RESULTS

Oxygen dissociation curves, generated at 41°C and 5% CO_2 (mean pH 7.43 ± 0.04 [$n = 47$] and 7.46 ± 0.04 [$n = 49$] in summer and winter, respectively), for summer- and winter-acclimatized juncos are given in Figure 1. Summer and winter O_2DCs did not vary significantly ($P >$ 0.05) in either of the Weibull parameters (B_0: t = 1.30, B_1: $t = 1.62$; df = 42). P_{50}'s for summer- and winter-acclimatized juncos were 54.1 torr and 54.0 torr, respectively, at 41°C and pH 7.5.

Hill plots derived from O_2DCs for summer- and winter-acclimatized juncos were not linear
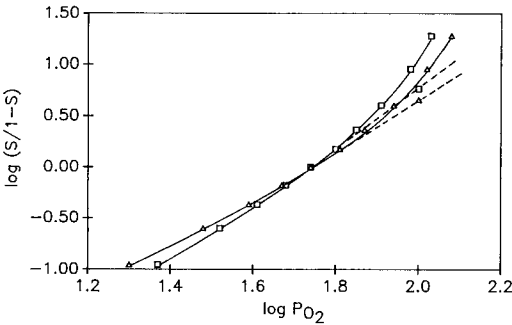
FIGURE 2. Hill plots derived from the oxygen dissociation curves in Figure 1 for summer-(triangle) and winter-acclimatized (square) juncos. Note the increasing n-values (slopes) at high saturations. The dashed lines correspond to a projection of the n-values around P_{50} to higher saturations for summer (triangle) and winter (square) birds.

(Fig. 2) as slope and the corresponding Hill coefficient (n = Δlog[%Sat/100 − %Sat]/Δlog P_{O_2}) increased with increasing saturation. At low saturations, below 20%, n-values for summer and winter were 2.0 and 2.3, respectively, and n-values around P_{50} were 2.5 in summer and 2.9 in winter. At saturations exceeding 80%, n was greater than 4.0, and n increased to greater than 5.4 above 90% saturation.

The effect of pH on the O_2DC was determined in spring and fall as the CO_2 Bohr effect. The combined mean Bohr coefficient (ϕ = Δlog P_{50}/ΔpH) from spring and fall for the Dark-eyed Junco was −0.46. This value is consistent with those recorded from other birds, which range from −0.39 to −0.67 (Baumann and Baumann 1977, Palomeque et al. 1980).

Seasonal mean values for hematocrit varied significantly [47.2 ± 3.3% in summer; 52.6 ± 2.0% in winter; P < 0.001]. Oxygen carrying capacity means were 13.35 ± 1.15 vol % and 14.48 ± 1.20 vol % in summer and winter, respectively. These values were also significantly different (P < 0.05).

DISCUSSION

Oxygen affinity and O_2DC shape of the blood of the Dark-eyed Junco did not vary seasonally. Thus, under standard conditions, oxygen unloading does not appear to be enhanced in winter as a result of a decrease in oxygen affinity. This is not completely surprising since inositol pentaphosphate, the principal organic phosphate controlling oxygen affinity in avian blood, is met-

abolically unreactive (Lutz 1980). However, oxygen affinity has been shown to decrease in some birds upon altitude acclimation (Bouverot et al. 1976, Black et al. 1978). Apparently, mechanisms capable of altering oxygen affinity in response to environmental conditions are invariant during seasonal acclimatization in the junco.

Although no seasonal change in hemoglobin oxygen affinity was apparent under standard conditions, a functional change in oxygen affinity may occur as a result of seasonal differences in conditions at the tissues. Increased muscular activity associated with sustained shivering in winter, without a corresponding increase in blood flow, could result in conditions at the tissues which would tend to decrease oxygen affinity and enhance unloading, irrespective of seasonal stability in blood oxygen affinity under standard conditions. Indeed, arteriovenous differences in P_{O_2} can vary profoundly with exercise at essentially the same oxygen carrying capacity (Nunn 1987). However, arteriovenous P_{O_2} differences in the pekin duck showed no variation upon cold exposure (−20°C) even though oxygen consumption increased 2.4 times above thermoneutral rates (Bech et al. 1984).

Under standard conditions of 41°C and pH 7.5, oxygen affinity of the blood of the Dark-eyed Junco (P_{50} = 54 torr) was lower than that for most other birds, and was at the upper end of the range of P_{50}s reported for other passerine birds (Palomeque et al. 1980). The Hill plot for junco blood showed increasing Hill coefficients with increasing saturation: n-values exceeding 4.0 at saturations above 80%. This pattern has been recorded for several other birds (Lutz 1980, Lapennas and Reeves 1983, Johansen et al. 1987). It is contrary to the situation in mammals, where Hill coefficients decrease at high and low saturations. Hill plots of this shape denote increased hemoglobin cooperativity as saturation increases, with very high cooperativity at high saturations. This allows high oxygen saturations to occur at relatively low partial pressures of oxygen. While an O_2DC with a high, constant, n-value would also result in efficient oxygen loading at relatively low P_{O_2}s, it would presumably result in higher oxygen saturation at P_{O_2}s characteristic of the tissues and thereby provide less efficient oxygen unloading to the tissues. The type of O_2DC found in the junco may be of great importance to animals with hemoglobins having low affinities for oxygen, allowing them to reach high sat-

uration at physiologically relevant P_{O_2}s and effectively unload oxygen to the tissues (Johansen et al. 1987).

For example, assuming an arterial P_{O_2} of 95 torr, the value claimed for the pigeon *Columba livia* (Bouverot et al. 1976), oxygen saturation of junco blood at 41°C and pH 7.5 would be 92.4% in winter and 88.7% in summer. If Hill coefficients around P_{50} are extended to 95 torr, oxygen saturation would become 87.4% in winter and 83.3% in summer. Thus, the pattern of increasing cooperativity with increasing saturation results in relative improvements in O_2 binding of 5.7% in winter, and 5.3% in summer. This allows for a corresponding increase in oxygen delivery which might be significant to small birds with high metabolic rates like the Dark-eyed Junco. The mechanisms responsible for this type of Hill plot are unknown, but may involve aggregation or association of hemoglobin molecules, thus modifying binding capabilities (Lapennas and Reeves 1983).

Hematocrit and oxygen carrying capacity were increased in winter-acclimatized juncos relative to summer juncos: hematocrit by 11.1% and O_2Cap by 8.6%. This adjustment functionally provides the same effect as a decrease in oxygen affinity: an increase in oxygen available to metabolizing tissues. Hematocrit has also been shown to increase in winter in American Goldfinches (*Carduelis tristis*) from Michigan (Carey and Morton 1976) and in White-crowned Sparrows (*Zonotrichia leucophrys*) from eastern Washington (deGraw et al. 1979). A winter increment of hematocrit in the junco presumably reflects increased erythropoiesis. Hematocrit could also be increased by reduction of plasma volume, but this would entail redistribution of body water among body compartments, since total body water as a percentage of lean wet body mass does not appear to vary seasonally in the junco (Helms et al. 1967).

Winter increases in hematocrit and O_2Cap may be energetically advantageous in assisting support of elevated winter oxygen demands. An increase in O_2Cap allows more oxygen to be delivered to the tissues per unit blood. Consequently, reduced blood flow rates may be required for delivery of a given amount of oxygen to the tissues, thus decreasing metabolic costs associated with pumping of blood. However, elevated hematocrits result in increased blood viscosity which would increase cardiac work and partially offset energetic advantages associated with reduced blood flow requirements. Nevertheless, augmentation of oxygen delivery to tissues by increased hematocrit and O_2Cap is probably energetically more economical than simply increasing blood flow and would seem better suited to increase the capacity for shivering and cold endurance.

Further study is needed to determine if seasonally fixed oxygen affinities are characteristic of other passerines, especially those inhabiting regions with harsher winters than western Oregon, and to determine the extent to which seasonally changing tissue conditions influence oxygen affinity and oxygen unloading at the tissues. However, these data indicate that *J. hyemalis* deals with increased winter demands for oxygen, at least in part, by increasing the blood oxygen carrying capacity while oxygen affinity, under standard conditions, remains constant. This pattern concurs with other evidence (Isaacks et al. 1982) suggesting that oxygen transport in birds may be less plastic than that of mammals, with reference to altering hemoglobin oxygen affinity in response to changing oxygen demands. If so, birds must employ other mechanisms for increasing oxygen availability under hypoxic conditions. Accordingly, the response observed in the junco may be characteristic of the general syndrome of seasonal acclimatization of vascular oxygen transport in small passerine birds.

ACKNOWLEDGMENTS

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