Constraints on vertebrate athleticism: The temperature dependence of red blood cell volume

The ability to perform at high levels of aerobic activity (i.e. athletic ability) increases with temperature among vertebrates. These differences in species' activity levels, from highly active to sedentary, are reflected in their ecology and behavior. Yet, the changes in the cardiovascular system that allow for greater oxygen supply rates at higher temperatures, and thus greater activity levels, remain unclear. Here we show that vertebrates provide more oxygen to tissues at higher temperatures in part by increasing the total volume of red blood cells in the body. Across 60 species of vertebrates (fishes,amphibians, reptiles, birds and mammals), whole-body red blood cell volume increases exponentially with temperature after controlling for effects of body size and taxonomy. These changes are accompanied by increases in relative heart mass, an indicator of athletic ability. The results help explain how temperature-dependent changes in cardiovascular design allow species to overcome the constraints of passive diffusion on oxygen supply.

Constraints on vertebrate athleticism: The temperature-dependence of red blood cell volume

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1. INTRODUCTION

The tremendous variation in athleticism among vertebrates is partially explained by differences in body temperature (Bennett & Ruben, 1979; Bennett 1987). Colder-bodied species tend to be more sluggish or sedentary, whereas warmer-bodied species tend to show higher levels of aerobic activity. From an ecological perspective, such temperature-dependent differences in activity underlie many differences in species' lifestyles, including feeding modes, movement patterns and rates of locomotion (Bennett, 1980; Filho et al. 1992; Angilletta, Huey & Frazier, 2010; Hein, Hou & Gillooly, 2012). From an evolutionary perspective, temperature-dependent increases in aerobic activity may also lead to greater fitness (Kingsolver & Huey 2008; Angilletta, Huey & Frazier, 2010). For this reason, the greater aerobic capacity afforded by higher temperatures is often cited as an explanation for the evolution of endothermy (Bennett & Ruben, 1979; Clarke & Portner, 2010).

Yet, we have much to learn about how organisms increase oxygen supply rates at higher temperatures (Hillman, Hancock & Hedrick, 2013). Over the short term, the exponential increase in oxygen consumption rate with temperature is presumably matched by increases in oxygen supply rate due to temperature effects on biochemical kinetics and related dynamics (e.g., increasing heart rate) (Gillooly et al., 2001; Kingsolver & Huey, 2008). However, over the longer term or during more intense exercise, species must overcome the weak temperature dependence of passive diffusion relative to oxygen consumption. For this reason, biologists have long debated whether aerobic activity levels are limited by diffusion (Wagner et al., 1990; Farrell, 2002; Gjedde, 2010).

Here we examine whether whole body red blood cell volume (not individual cell volume), a key indicator of oxygen carrying capacity, increases with temperature across species. Whole body red blood cell volume, which is typically expressed as a percentage of body mass (i.e. ml/100 g body mass), varies by more than an order of magnitude across vertebrates for reasons that remain unclear (Bond & Gilbert, 1958; Thorson, 1961; Thorson, 1968). The total volume of red blood cells in a species is often considered to be independent of body mass, and greater in species with higher aerobic activity levels (Hillman, 1976; Prothero, 1980; Filho et al, 1992; Gallaugher & Farrell, 1998; Dawson, 2005), but there is no known relationship with temperature. In addition, we examine if/how differences in red blood cell volume are related to differences in relative heart mass (i.e., heart mass/body mass x 100). Relative heart mass is also greater in more athletic species (Farrell, 1991; Bishop, 1999; Vinogradov & Anatskaya, 2006). In both analyses, we control for effects of body size and taxonomy. These analyses provide a step toward understanding broad-scale differences in vertebrate athleticism based on temperature-dependent changes in cardiovascular design.

2. METHODS

(a) Data

Data on red blood cell volume (ml/100 g body mass) were compiled for vertebrates from a range of habitat types (freshwater, marine, terrestrial), and span a broad range of body sizes, temperatures, taxonomic affiliations, and athletic abilities (Supplementary Materials, Appendix 1). Data were originally collected using indicator dilution or labeling methods (Zierler, 2000). Analyses were restricted to adult or sub-adult individuals as values may change through early ontogeny (Garland & Else, 1987). Analyses also did not include *i*) air-breathing divers (e.g.

marine mammals, sea turtles) given their exceptional nature (Costa, Gales & Crocker, 1998), *ii*) elasmobranchs given the confounding effects of low albumin concentration on measurement (Tort et al., 1991), *iii*) Antarctic fish with little or no hemoglobin, and *iv*) and urodeles given the large fraction of blood cells held in the spleen (see discussion). To the best of our knowledge, all other available data were included in the analyses.

Estimates of relative heart mass were taken from studies on adult individuals of the same species. On occasion, heart mass was estimated from ventricular mass assuming ventricle mass comprised 60 % of heart mass (Santer, 1980; Seymour, 1987; Brill & Bushnell, 1991).

Body mass estimates from the original study of red blood cell volume were used. Body temperatures were estimated using the resting body temperatures of endotherms (birds and mammals), and the preferred body temperatures of ectotherms (amphibians, reptiles, and fishes). In some ectotherms, particularly with fishes, mean study or site temperature was used as a proxy measure of preferred temperature. Additionally, in a few cases where temperature estimates for a particular species were unavailable, the temperature of a species from the same genus was used.

(b) Analyses

To evaluate the body size and temperature dependence of red blood cell volume, and the dependence of red blood cell volume on relative heart mass, we first performed type II nested ANOVAS (Sokal & Rohlf, 1969) to account for possible effects of evolutionary relatedness among species. At present, the vertebrate phylogeny is undergoing major revision and no well-supported phylogeny exists (Thomson & Shaffer, 2010). In the absence of such a phylogeny, nested ANOVAS are the preferred method of analysis to address this issue (Harvey & Pagel, 1991).

With the nested ANOVAS, we examined the influence of taxonomic class, order within class, and family within order, to determine the appropriate taxonomic level for analysis for the variables in question. This analysis revealed that significant variation in the dependent variables (body size, temperature, and relative heart mass) can be explained at the level of taxonomic order ($p<0.05$). Thus, for both analyses, we fit weighted linear regressions using mean values at the level of taxonomic order. The regression is weighted depending on the proportion of taxa within each order. For the relationship between size, temperature and red blood cell volume, we fit a model of the form $\ln RBC = a\ln M + bT + \varepsilon + c$. Here RBC is red blood cell volume (ml/100 g body mass), a is a body-mass scaling exponent, b ($°C^{-1}$) characterizes the exponential temperature dependence, ε represents random error, and c is a taxon-specific constant. The variables M and T in this formulation are mean values of body mass (g) and temperature (°C) at the order level. Similarly, to describe the relationship between red blood cell volume (RBC) and relative heart mass (H), we fit a model of the form $\ln(RBC) = a\ln(H) + \varepsilon + c$ using the mean values at the level of order. Then, to further investigate the effects of taxonomic order on our results, we performed a bootstrap analysis on each linear model. The bootstrap analysis consisted of resampling taxa within each order and recalculating the mean values for each of the variables in the model (Efron & Tibshirani, 1993). Weighted linear regressions were then estimated with these new values after 30,000 repetitions produced consistent estimates.

To graphically represent the effects of body size and temperature on red blood cell volume, the dependent variable was divided by the observed mass-dependence (i.e. $y = \ln[RBC/M^a]$), and then this new "body-mass-corrected" value, y, was plotted against temperature for values at the level of order. To statistically evaluate the relationship between relative heart mass and red blood cell volume, weighted least squares regression was performed on log

transformed data at the level of order. In the plots, we show all species-level data but show only the lines fit to data at the order level.

3. RESULTS AND DISCUSSION

Red blood cell volume (RBC; ml/100 g body mass) varied with both body mass and temperature across the 60 species considered here (birds: n = 7; mammals: n = 13; reptiles: n = 14; amphibians: n = 6; fishes: n = 20). Together, the two variables explained 89.5% of the variation in RBC (range: 0.6-6.8) based on weighted multiple regression of log-transformed data ($F = 93.79$, 2 and 22 d. f., $P < 0.0001$) at the level of order. Both showed significant, independent effects on RBC ($P < 0.001$). RBC decreased with body mass, M, as $RBC \propto M^{-0.06}$ (95% CI: -0.07 to -0.04), and increased exponentially with temperature, T (°C), as $RBC \propto e^{0.06T}$ (95% CI: 0.060 to 0.068 $°C^{-1}$). Figure 1 shows a plot of the natural logarithm of body-mass-corrected RBC versus temperature ($y = 0.064T - 0.94$). Results from the bootstrap analyses show the same relationship of red blood cell volume to body size and temperature (Fig. 1). On average, a 20 ºC increase in temperature results in a 3.5-fold increase in blood volume.

Results shown in Figure 1 provide insights into how vertebrates increase oxygen supply rates at higher temperatures to support higher activity levels. They suggest that vertebrates not only increase heart rate at higher temperature, but that they also increase the total volume of oxygen-carrying red blood cells to meet oxygen demands. Moreover, our results show that red blood cell volume is higher in smaller-bodied species, which tend to show greater levels of aerobic activity (Farrell, 1991; Bishop, 1999). Across species, red blood cell volume also increased systematically with relative heart mass (Figure 2), albeit less than linearly. We

speculate that this may be related to differences in blood viscosity (i.e., hematocrit) and the possible effects of such differences on heart size (Farrell, 1991; Bishop, 1999).

Red blood cell volume was also correlated with relative heart mass ($F = 28.69$, 1 and 19 d. f., $P < 0.0001$) among the 30 species considered here (birds: n= 7; mammals: n = 9; reptiles: n = 6; amphibians (anurans): n = 4; bony fishes: n = 5). The systematic increase in red blood cell volume with relative heart mass observed in our regression model ($y = 0.67x+1.38$; $r^2 = 60\%$; n = 20) was also observed using the bootstrap technique ($y = 0.64x+1.39$; Fig. 2).

Previous work has reported that relative heart mass, and other structural attributes of cardiovascular systems important to oxygen supply, show about the same size and temperature dependence as that of red blood cell volume. (i.e., blood gas barrier thickness; Gillooly, unpublished data). These structures may all vary in proportion to maximum oxygen consumption rate (i.e. $V0_2$ max) if organisms are structured to perform best at these levels, as has been suggested for mammals (Weibel et al., 2004). Interestingly, the body mass and temperature dependence of red blood cell volume is strikingly similar to that reported for maximum oxygen consumption in some groups (Bishop 1999; Weibel et al. 2004).

Note, however, that limitations on oxygen availability and related changes to red blood cell volume may come in many forms-not just through activity (e.g., the cost/mode of locomotion, hypoxia, and elevation) (Carey & Morton, 1976; Bennett & Ruben, 1979; Farmer, 1999; Hillman, Withers & Drewes, 2000). In urodeles, for example, a large fraction of red blood cells may be held in the spleen and only released at high temperatures to guard against hypoxia (Tort et al., 1991). In these cases, measurement of circulating red blood cell volume at preferred temperatures would not be comparable to other vertebrates. Moreover, to some degree, cardiovascular design is phenotypically plastic. Species including our own are known to increase blood volume with exercise (Lillywhite & Smits, 1984; Convertino, 1991; Birchard, 1997).

Together, these results provide an example of how a structural feature in cardiovascular systems may vary with temperature and affect aerobic activity levels. It remains unclear whether the size of other structures may be similarly temperature-dependent (e.g., alveolar diameter, capillary density) given the limited research on this subject, but if so, this could change our understanding of oxygen supply dynamics. Specifically, it could lead us to reconsider the importance of diffusion limitation in system-level oxygen supply models (e.g., Spatz, 1991; Dawson 2005).

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Figure 1

The natural logarithm of body mass-corrected red blood cell volume vs. temperature in vertebrates.

Figure 1 :The natural logarithm of body mass-corrected red blood cell volume (i.e., ln (RBC/M^{a}) vs. temperature (^{o}C) in vertebrates. Red blood cell volume is a whole-body measure expressed as ml/100 body mass. The regression line shown is based on weighted values for data averaged at the level of taxonomic order to address phylogenetic non-independence. Bootstrapping analysis produced a similar line (y = 0.064x- 0.95; see methods).

Figure 1, Gillooly and Zenil-Ferguson

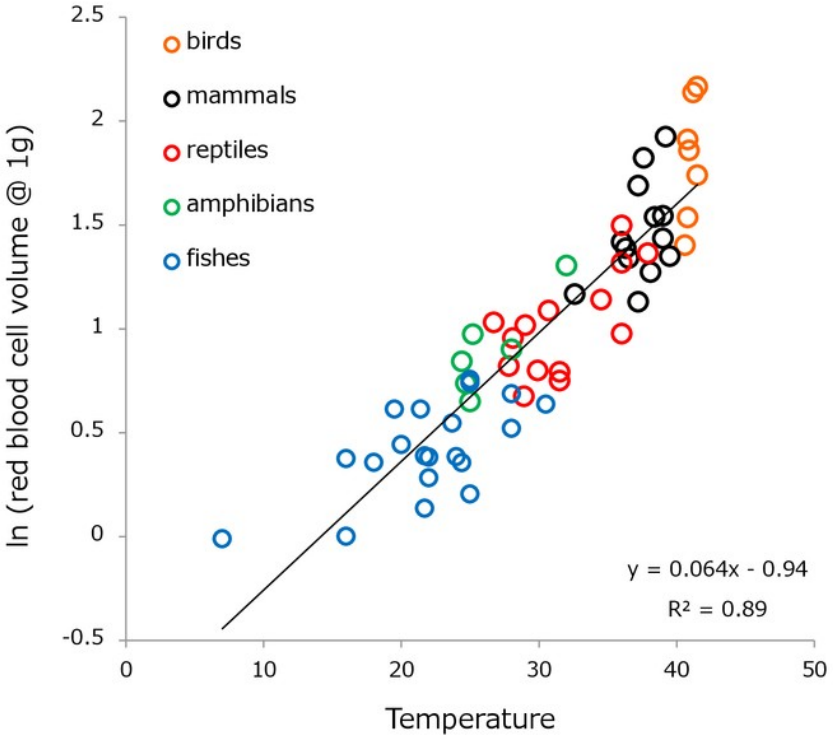

Figure 2

The natural logarithm of red blood cell volume vs. the natural logarithm of relative heart mass in vertebrates.

Figure 2 :The natural logarithm of red blood cell volume (ml/100g body mass) vs. the natural logarithm of relative heart mass (heart mass/body mass x 100) in vertebrates. The regression line shown is based on weighted values for data averaged at the level of taxonomic order to address phylogenetic non-independence. Bootstrapping analysis produced a similar line (y = 0.65x + 1.39; see methods).

Figure 2, Gillooly and Zenil-Ferguson