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 at higher temperatures, and thus greater activity levels, remain unclear. Here we show that the total volume of red blood cells in the body increases exponentially with temperature across vertebrates, 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 point to one way vertebrates may increase oxygen supply to meet the oxygen demands of greater activity at higher temperatures.

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

James F. Gillooly* and Rosana Zenil-Ferguson
Department of Biology, University of Florida, Gainesville, FL 32611 USA
*author to whom correspondence should be addressed (email: gillooly@ufl.edu)

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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, 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 lead to greater fitness (Kingsolver & Huey 2008; Angilletta, Huey & Frazier, 2010). For this reason, the greater aerobic activity 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 the relationships between temperature, activity level, and oxygen supply/demand (Clarke & Portner, 2010; Hillman, Hancock & Hedrick, 2013). At moderate levels of activity, temperature effects on biochemical kinetics and related dynamics (e.g., increasing heart rate) allows species the ability to both consume more oxygen and supply more oxygen, and thus sustain greater activity (Gillooly et al., 2001; Kingsolver & Huey, 2008). However, more prolonged or intense activity is generally considered to be limited by oxygen supply (di Prampero, 1985). Temperature effects on biochemical kinetics may not be sufficient to meet high oxygen demands (Wagner et al., 1990; Farrell, 2002; Gjedde, 2010).

Here we examine if total red blood cell volume (not individual cell volume) increases with temperature across species to better meet oxygen demands. The percentage of body mass comprised of red blood cells (i.e., *RRBC*) varies by more than an order of magnitude across vertebrates for reasons that remain unclear (Bond & Gilbert, 1958; Thorson, 1961; Thorson, 1968). *RRBC* 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. If *RRBC* does increase with temperature, and this coincides with increases in cardiac output, then species could consume more oxygen at higher temperatures, as suggested by Fick's Principle (Hillman, 1976). Note that most oxygen-carrying hemoglobin is found in red blood cells, and the cellular density of hemoglobin varies little across species with different-sized cells (Hawkey et al., 1991).

In addition, we examine if/how differences in *RRBC* may be related to differences in relative heart mass when described as a percentage of body mass. Relative heart mass, like *RRBC*, tends to be greater in more athletic species (Farrell, 1991; Bishop, 1999; Vinogradov & Anatskaya, 2006). A relationship between heart mass and red blood cell volume would suggest increases in cardiac stroke volume with temperature. In both analyses, we control for the effects of body size and taxonomy. These analyses provide a step toward better understanding how species meet the oxygen demands at higher temperatures to support higher activity levels.

2. METHODS

(a) Data

Data on *RRBC* were compiled from published sources 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), and originally expressed as either percent body mass or ml RBC/100 g body mass. These units are equivalent assuming a specific gravity of 1 (Trudnowski & Rico, 1974). 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) and the difficulty in obtaining estimates of preferred body temperature. To the best of our knowledge, all other available data on *RRBC* were included in the analyses for which body size and temperature estimates were also available.

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 that ventricle mass comprised 60 % of heart mass (Santer, 1980; Seymour, 1987; Brill & Bushnell, 1991) (see Appendix 1).

Body mass estimates from the original studies of *RRBC* were used. For endotherms (birds and mammals), resting body temperatures were used, and assumed to be roughly equivalent to active body temperatures. For ectotherms (amphibians, reptiles, and fishes), the preferred environmental or body temperatures were used assuming these are the temperatures at which species are typically active. If temperature estimates for a particular species were unavailable, the temperature of one or more species (using mean value) from the same genus was used (see Appendix 1). In the case of fishes, the temperature at which a species was held was sometimes used as the preferred temperature (see Appendix 1).

(b) Analyses

To evaluate the body size and temperature dependence of *RRBC*, and the dependence of *RRBC* 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 variables for each analysis 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 *RRBC*, we fit a model of the form $\ln RRBC = a \ln M + bT + c$. Here a is a body-mass scaling exponent, b (°C^{-1}) characterizes the exponential temperature dependence, and c is a taxon-specific constant that includes random error. The variables M (g) and T (°C) in this formulation are mean values of body mass (g) and temperature (°C) at the order level. Similarly, to describe the relationship between *RRBC* and relative heart mass (*RHM*), we fit a model of the form $\ln (RRBC) = a \ln (RHM) + c$ 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 *RRBC*, we divided *RRBC* through by the observed mass-dependence, and then the natural logarithm of this new "body-mass-corrected" value (i.e. $y = \ln [RRBC/M^{a}]$) was plotted against temperature for values at the order level. To statistically evaluate the relationship between *RHM* and *RRBC*, weighted least squares regression was performed on ln-transformed data at the level of order. In the plots, we present all species-level data, but lines are only fitted to the data at the order level.

3. RESULTS

RRBC varied with both body mass (M, in grams) and temperature (T, in °C) across the 60 species considered here (birds: n = 7; mammals: n = 13; reptiles: n = 14; amphibians: n = 6; fishes: n = 20) following the equation: $\ln (RRBC) = -0.066 \ln (M) + 0.063T - 0.88$. Together, the two variables explained 85.3% of the variation in *RRBC* (range: 0.6-6.8) based on weighted multiple regression of ln-transformed data (F = 63.85, 2 and 22 d. f., $P < 0.0001$) at the level of order. Both showed significant, independent effects on *RRBC* ($P < 0.001$). As indicated by the equation, *RRBC* decreased with body mass, *M (g)*, as $RRBC \propto M^{-0.06}$ (95% CI: -0.08 to -0.04), and increased exponentially with temperature, *T* (°C), as $RRBC \propto e^{0.06T}$ (95% CI: 0.058 to 0.068 °C^{-1}). Figure 1 shows a plot of the natural logarithm of body-mass-corrected *RRBC* (i.e., ln [RRBC $M^{-0.06}$] versus temperature ($y = 0.063T - 0.88$). Based on the fitted line, *RRBC* increases by about an order of magnitude across species from 5- 40 °C, after accounting for the effects of body mass.

RRBC was also positively correlated with relative heart mass (Figure 2; F = 36.42, 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

RRBC with relative heart mass observed in our regression model (y = 0.72 RHM+1.41; r^2 = 65.7%; n = 20) was similarly observed using the bootstrap technique (y = 0.70 RHM +1.43; Fig. 2). We note, however, that the increase in *RRBC* with relative heart mass was less than proportional (i.e., slope < 1).

4. DISCUSSION

The results shown here provide insights into how vertebrates meet the oxygen demands of greater activity at higher temperatures. In addition to increasing heart rates at higher temperatures, these results show that vertebrates also increase the total volume of oxygen-carrying red blood cells. The observed increase in *RRBC* with relative heart mass points to an increase in stroke volume at higher temperatures. However, the observed increase in *RRBC* with temperature (i.e., Q_{10} of approx. 1.9) is less than the temperature-dependence of oxygen consumption described for most resting vertebrates (i.e., Q_{10} of approx.. 2.7 by Gillooly et al. 2001). This difference may indicate that the temperature-dependence of oxygen consumption is weaker at high activity levels, or alternatively, that other changes in the cardiovascular system increase the temperature-dependence of oxygen supply. Many factors that affect oxygen supply rate may change to some degree with temperature (e.g., blood viscosity, oxygen dissociation; Sidell, 1998). In some cases, factors such as stroke volume and heart rate may offset each other to some degree in determining oxygen supply rate (Machida & Aohagi 2001). Thus, a better understanding of how all relevant factors vary with size and temperature, in the context of Fick's principle, would help to elucidate constraints on oxygen supply at the whole organism level.

Similarly, many factors aside from temperature may affect *RRBC* (e.g., the cost/mode of locomotion, hypoxia, and elevation) (Carey & Morton, 1976; Bennett & Ruben, 1979; Farmer, 1999; Hillman, Withers & Drewes, 2000). Higher *RRBC*, for example, may simply be a means to guard against hypoxia. In urodeles, a large fraction of red blood cells may be held in the spleen and only released at high temperatures for just this reason (Tort et al., 1991). This highlights the fact that, to some degree, *RRBC* is phenotypically plastic. For example, species including our own may increase blood volume with exercise (Lillywhite & Smits, 1984; Convertino, 1991; Birchard, 1997).

Thus, the body size and temperature dependence of *RRBC* shown here raise interesting questions regarding the relationship between temperature and cardiovascular design. The dependencies for *RRBC* are quite similar to those shown for 2 other cardiovascular structures important to oxygen supply- relative heart mass and blood gas barrier thickness (Gillooly, unpublished data). It remains to be seen whether other structures are similarly size and temperature-dependent (e.g., alveolar diameter, capillary density)-there has been little research in this area. If so, this could alter our understanding of system-wide oxygen dynamics. To some degree, our current understanding are based on models that do not consider temperature (e.g., Spatz, 1991; Dawson 2005).

The body size and temperature dependence of *RRBC* also raise interesting questions regarding differences in athleticism or activity level among vertebrates . Both "athleticism" and "activity level" are frequently used as descriptors (e.g., Bishop, 1999), but these descriptors may not equate to standard measures of aerobic performance (e.g., V_{02} max or aerobic scope). For example, high activity over prolonged periods, which a species may exhibit in its natural habitat, may or may not be captured by these measures (Peterson et al., 1990). That said, the body mass and temperature dependencies of *RRBC* are strikingly similar to those reported for maximum oxygen consumption in some groups (Bishop 1999; Weibel et al. 2004). Thus, our results appear consistent with the hypothesis that natural selection acts on the design of cardiovascular systems to maximize oxygen supply rates during high aerobic activity (Weibel et al., 2004).

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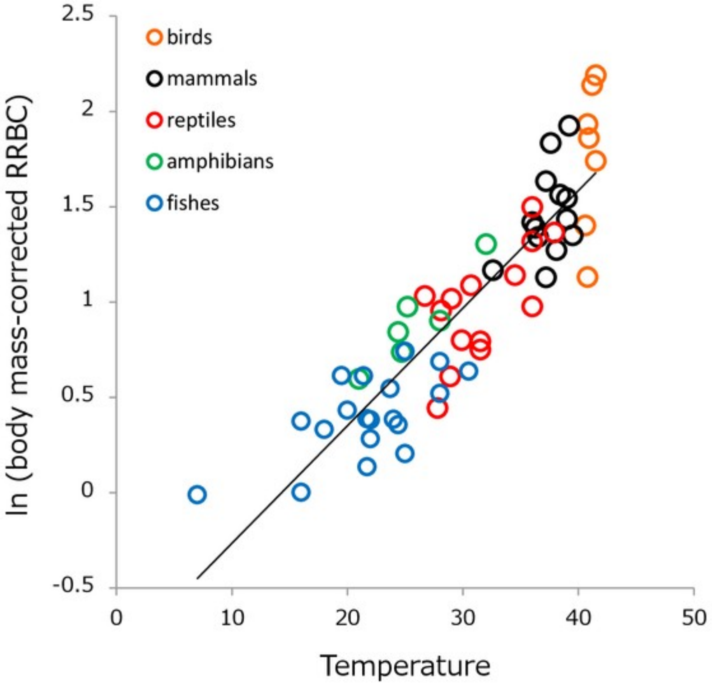
Figure 1

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

Figure 1 : The natural logarithm of body mass-corrected relative red blood cell volume vs. temperature in vertebrates. The regression line shown (i.e., ln $(RRBC/M^{-0.06}) = 0.063T-0.88$; $R^2 = 85.3$) is based on weighted values for data averaged at the level of taxonomic order to address phylogenetic non-independence. Relative red blood cell volume (RRBC) is expressedas a percentage of body mass, temperature (T) is expressed in degrees Celsius, and body mass (M) is expressed in grams. Bootstrapping analysis produced a nearly identical line (see methods).



2.5
2
1.5
1
0.5
0
-0.5
ln (body mass-corrected RRBC)
0
10
20
30
40
50
Temperature
○ birds
○ mammals
○ reptiles
○ amphibians
○ fishes

Figure 2

The natural logarithm of relative redblood cell volume vs. the natural logarithm of relative heart mass invertebrates.

[p] **Figure 2** : The natural

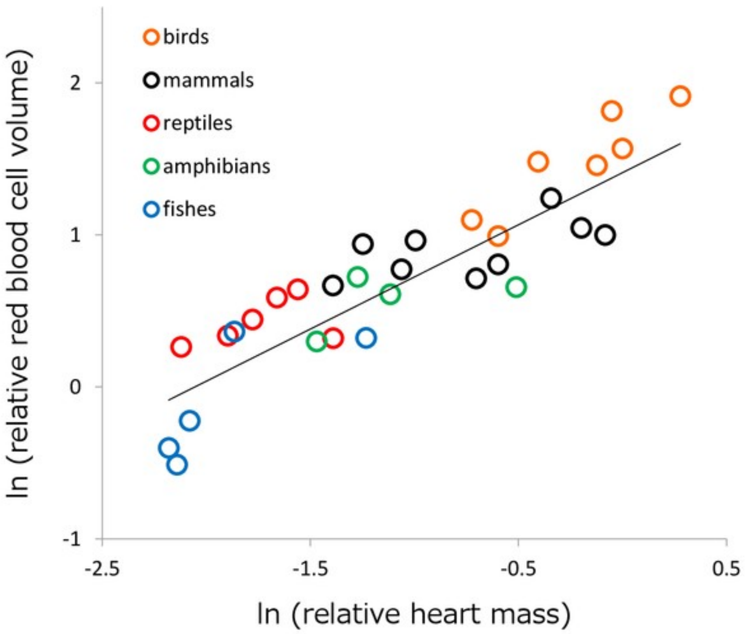
logarithm of relative red blood cell volume (% body mass) vs. the natural logarithm

of relative heart mass (% body mass) in vertebrates. The regression line shown (i.e.,

ln (RRBC) = 0.72 RHM +1.41; $R^2 = 65.7$) 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 ($y = 0.70x + 1.43$; see methods). [p]


birds
mammals
reptiles
amphibians
fishes
ln (relative red blood cell volume)
2
1
0
-1
-2.5
-1.5
-0.5
0.5
ln (relative heart mass)