The Relationship between Mammalian Body Mass and the Oxygen Carrying Capacity of Blood

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It is generally accepted that mass-specific metabolic rate scales negatively with body mass. This implies that the amount of oxygen delivered per gram of body mass scales similarly, which suggests that oxygen carrying capacity in blood may also decrease with increasing body mass. To investigate this hypothesis, we measured the hemoglobin concentration of four mammalian species that differ significantly in body mass: sheep, rabbit, rat, and mouse. We did not find any meaningful trend between the mean hemoglobin concentration in blood and body mass. For example, sheep had a hemoglobin concentration (14.9±1.3 g Hb/100mL blood) which was not significantly different from that of mouse (14.3±0.6 g Hb/100mL blood). Thus, the data from our experiment did not support our hypothesis. We attribute this unexpected result to experimental error and small sample size. In this study, we also measured red blood cell count and hematocrit, and calculated mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), and mean cell hemoglobin (MCH). However, we did not find any meaningful trends, suggesting that body mass has no consistent effect on how hemoglobin is packaged between species. Further research is required to make more definitive conclusions about the relationship between metabolic rate and body size in mammals.

<u>Introduction</u>

Metabolic rate is a measure of an organism's energy expenditure (Moyes and Schulte 2016 p. 628). While it is most closely linked to the rate of ATP production and usage, metabolic rate is more often approximated by measures of rates of oxygen consumption, carbon dioxide production, and heat production, since all of these variables are substrates or byproducts of metabolism (Moyes and Schulte 2016 p. 628-629).

It has been established that a positive allometric relationship exists between net metabolic rate and body mass (Moyes and Schulte 2016 p. 629). This relationship is logical, since the amount of energy required for basic bodily functions should increase with additional body mass. Although the exact value of the scaling exponent for metabolic rate varies, with estimates for different animal taxa placing it between 0.64 and 0.75, the general consensus is that it is less than 1 (Moyes and Schulte 2016 p. 629; White *et al*. 2006 p. 126). Therefore, mass-specific body weight scales negatively with body weight: larger animals have lower metabolic rates and require less oxygen per mass of body tissue. This suggests that the amount of oxygen required to be delivered per gram of tissue is decreased, and leads to our instructor Agnes Lacombe's hypothesis that the ability of blood to carry oxygen will decrease with increasing body mass.

In this study, we compare the oxygen carrying capacities of blood samples obtained from four mammalian species of differing body mass (sheep, rabbit, rat, and mouse, in order of decreasing body mass). Oxygen carrying capacity is defined as the maximum quantity of oxygen that a given volume of blood can hold, which includes oxygen carried by respiratory pigments such as hemoglobin, as well as any oxygen dissolved in the blood (Moyes and Schulte 2016 p. 473). However, dissolved oxygen only comprises a negligibly small amount of oxygen carrying capacity under normal conditions, and so oxygen carrying capacity is most accurately estimated by hemoglobin concentration. We will determine hemoglobin concentration by comparing the optical properties of solutions containing our blood samples to solutions whose hemoglobin content is known.

In addition to oxygen carrying capacity, we will also consider how hemoglobin is packaged in the blood of each of our four species, by determining the relative and absolute quantities of red blood cells in whole blood (using hematocrit and red blood cell counts, respectively), and through MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration), which are estimates of the hemoglobin content of individual red blood cells.

<u>Material and Methods</u>

Subjects

The experiment was done using blood from four different species of varying sizes to test the effects of body mass on oxygen carrying capacity. The four species used were mice, rats, rabbits, and sheep, the sample sizes were three, five, six and three respectively. All samples were taken from healthy adult, female subjects. The sheep were 69-76kg, rabbit were 2.7-4.1kg, rats were 450-510g, and mice 30-32g. Each species was raised at sea level in the Lower Mainland and were therefore acclimatized to approximately the same partial pressure of oxygen in the atmosphere. This was necessary to control for potential altitude-based differences in blood

composition (Moyes and Schulte 2016, p. 361). The experiment was blind so groups were given a blood sample labelled A, B, C, or D. It was later revealed that species A were the sheep; species B were the rabbits, species C, the rats, and species D, the mice.

Design and Protocol

We conducted a count of the red blood cells present per mm^3 of whole blood for each of the four mammalian species. The number of red blood cells were counted using a hemocytometer and a high powered microscope. Each student independently produced their own blood dilution and obtained two counts for that dilution, which were averaged. The two students working on each blood sample compared their values, which were considered acceptable if they were within 10% of each other; otherwise, resource-permitting, they were discarded and the procedure repeated until the values fell within the acceptable range.

To determine the concentration of hemoglobin in blood, each pair of students vortexed a sample of their species blood mixed with Drabkin solution. Together, the vortex and presence of Drabkin solution broke the red blood cells and released hemoglobin into solution. We were then able to use a spectrophotometer to determine a percentage transmittance of the sample. A calibration curve was plotted and used to determine the concentration of hemoglobin in the blood.

Blood hematocrit, the proportion of red blood cells in blood, was determined by placing blood into capillary tubes and centrifuging the tubes. We then used rulers to measure the length of the red portion and the entire portion to determine the percent of hematocrit present in the blood. Depending on the quantity of blood available, 1 to 2 hematocrit measurements were obtained for each blood sample. Where 2 values were obtained, they were considered acceptable if they were within 2% of each other; otherwise, they were discarded and repeated until within the acceptable range, resource-permitting.

Blood cell indices, MCH, MCV and MCHC, were determined via calculations using RBC, hematocrit, and hemoglobin concentration. See "Data Analysis" for more details. For further details of the procedure and materials, refer to the lab manual of the hematology lab of Biology 363.

Statistical and Data Analysis

A maximum of three blood samples per species were available on each of the two days of experimentation. However, five of the data sets collected on January 21 were labelled as sheep data, and only one as mouse data, suggesting that two of the mouse data sets were mislabelled as sheep data. Since there is no way of knowing which of the sets of data are the incorrect ones, all sheep data that were taken on January 21 were excluded from analysis.

Additionally, the set of data collected by Fash and Lehn was excluded from analysis because the hemoglobin concentration recorded was 56.4g/100 mL blood. Assuming that hemoglobin concentration is normally distributed in rats, and using the mean and standard deviation calculated by excluding this data point, this extreme value is over 17 standard deviations away from the mean. Considering our small sample size, exclusion of this data point is justified to prevent the inflation of the mean to a value unlikely to be representative of the population.

Individual red blood cell was done by summing the amount of red blood cells in 5 squares on the hemocytometer and multiplying it by 10000 to give the # RBC/mm^3. Each pair of students performed a RBC count and supplied two to four values for their animal subject. Those values were averaged and then used for further statistical analysis.

Hemoglobin concentration was calculated by plotting the absorbance of the solution with unknown concentration on the calibration curve. Each pair of students supplied one value for the hemoglobin concentration for their animal subject, this value was used for further statistical analysis.

Measuring the length of the packed red blood cells and dividing it by the length of the whole blood column gives the amount of hematocrit, which was then converted into a percentage. Each pair of students supplied one value for the hemoglobin concentration for their animal subjects, this value was used for further statistical analysis.

The mean cell volume (MCV) is the average volume of individual red blood cells. Each group of students supplied one value for MCV, MCH and MCHC for their animal subject, this value was used for further statistical analysis. For example, with regard to mice MCV data, three groups provided values. The mean value for MCV was calculated by averaging those values.

$$MCV = \frac{Hematocrit}{\# \, RBC} = \frac{\frac{30}{100}uL}{4715000 \, RBC/mm^3} \times \frac{1 \, L}{10^6 uL} \times \frac{10^{15} fL}{L} = 63.6 \, fL$$

The mean cell hemoglobin concentration (MCHC) represents the average concentration of hemoglobin inside individual red blood cells.

$$MCHC = \frac{[Hb]}{Hematocrit} = \frac{11.85 \frac{g \, Hb}{100mL} blood}{30/100} = 39.5 \, g \, Hb/100mL \text{ of blood}$$

Mean cell hemoglobin (MCH) is the average hemoglobin content inside a red blood cell.

$$MCH = \frac{[Hb]}{\# \, RBC} = \frac{11.85 \, g \, Hb}{100 \, mL} \times \frac{mm^3}{4715000 \, RBC} \times \frac{1 \, mL}{1 \, cm^3} \times \frac{1 \, cm^3}{1000 \, mm^3} \times \frac{10^{12} pg}{1 \, g} = 25.1 \, pg$$

Significant differences were determined by calculating 95% confidence intervals (CI) for each mean value on excel. If there is any overlap of the confidence intervals, then the mean values will not be significantly different. If there is no overlap of the confidence interval, then there is a significant difference and we can reject the null hypothesis and accept the alternative hypothesis.

Results:

There is no clear pattern in hemoglobin concentration according to species mass (Figure 1). Rabbit blood had a hemoglobin concentration of 12.0±0.6 g/100mL blood, which was significantly smaller than that of sheep (14.9±1.3 g/100mL blood), rat (17.3±1.2 g/100mL blood), and mouse (14.3±0.6 g/100mL blood). In turn, mouse blood was significantly lower in hemoglobin concentration than rat blood. However, there was no significant difference between sheep blood and mouse blood, or between sheep blood and rat blood.

Neither is there any consistent trend for red blood cell concentration (Figure 2). Among rabbit, rat, and mouse blood, there appears to be a weak trend in red blood cell concentration: mouse - the species with the smallest body mass - has the greatest concentration (8.5±1.1 cells/pL); rat, which has a greater body mass than mouse, has a concentration of 7.1±0.5 cells/pL, which is smaller than the concentration of mouse blood, though not significantly so; and rabbit - the species with the larger body mass of the three - has a red blood cell concentration of 4.7±0.7 cells/pL, which is significantly lower than both rat and mouse blood. However, sheep blood does not fit this pattern, since it has the largest body mass but also has a red blood cell count (11.2±1.0 cells/pL) that is significantly greater than that of the other species studied.

There is no consistent trend between species mass and hematocrit (Figure 3). Rabbit hematocrit (32±3%) is significantly smaller than the other three species, but there are no significant differences among the hematocrit of sheep (40±1%), rat (43±3%), or mouse (43±2%).

Likewise, there is no clear pattern in MCH or MCV. When considering MCH (Figure 4) and MCV (Figure 5) for rabbit, rat, and mouse blood only, there seems to be a weak trend. Mouse MCH (17±2 pg) is significantly lower than rat MCH (24±3 pg), which is lower (though not significantly) than rabbit MCH (26±3 pg). Similarly, mouse MCV (51±5 fL) is lower (though not significantly) than rat MCV (60±7 fL), which is lower (though not significantly) than rabbit MCV (70±15 fL). However, sheep is the exception to all of this, as its MCH (13±0.4 pg) and MCV (36±3 fL) are significantly lower than all the other species.

There is no clear trend in MCHC (Figure 6). Mouse MCHC (33±2 g/100mL packed RBC) is significantly less than rat MCHC (41±2 g/100mL packed RBC). However, there are no significant differences between any of sheep MCHC (37±3 g/100mL packed RBC), rabbit MCHC (38±4 g/100mL packed RBC), or rat MCHC.
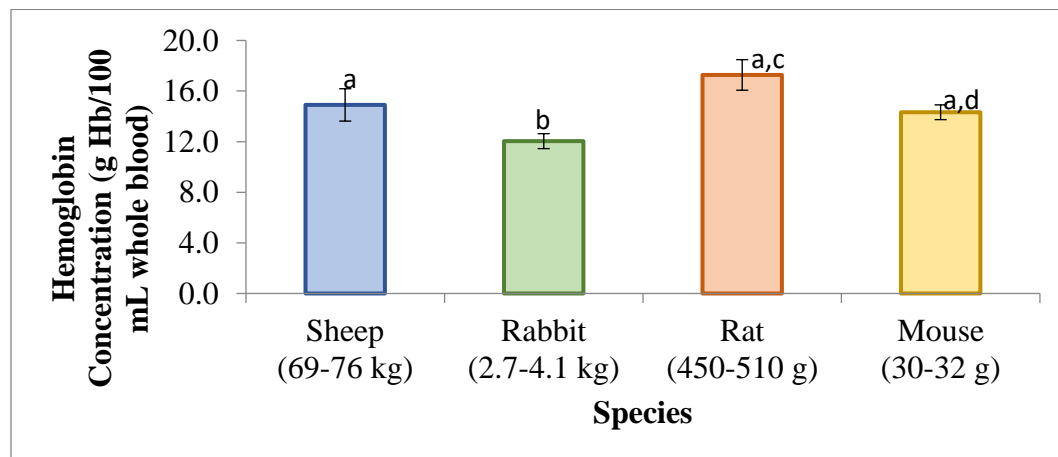

Figure 1: Mean Haemoglobin Concentration of Blood. For sheep n=3, n=6 for rabbits, n=5 for rats, and n=3 for mice. The error bars represent 95% confidence intervals and letters represent significant differences.
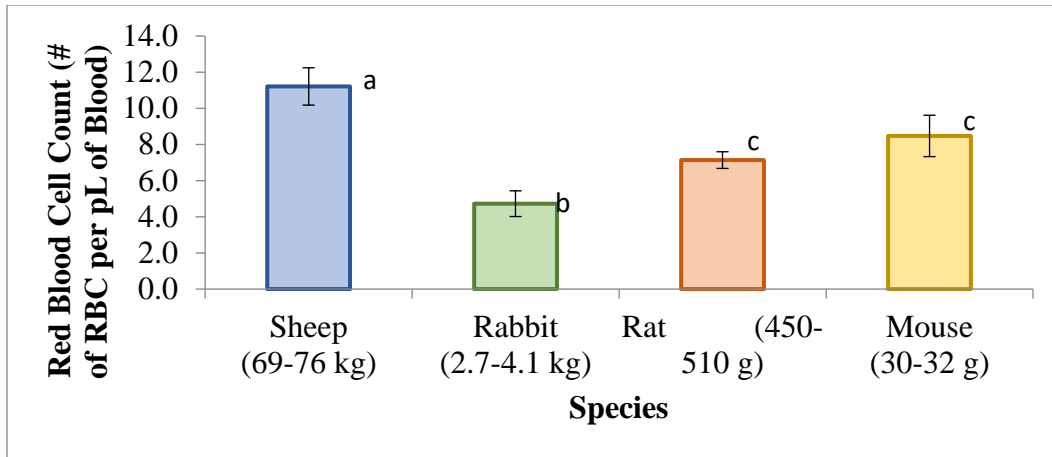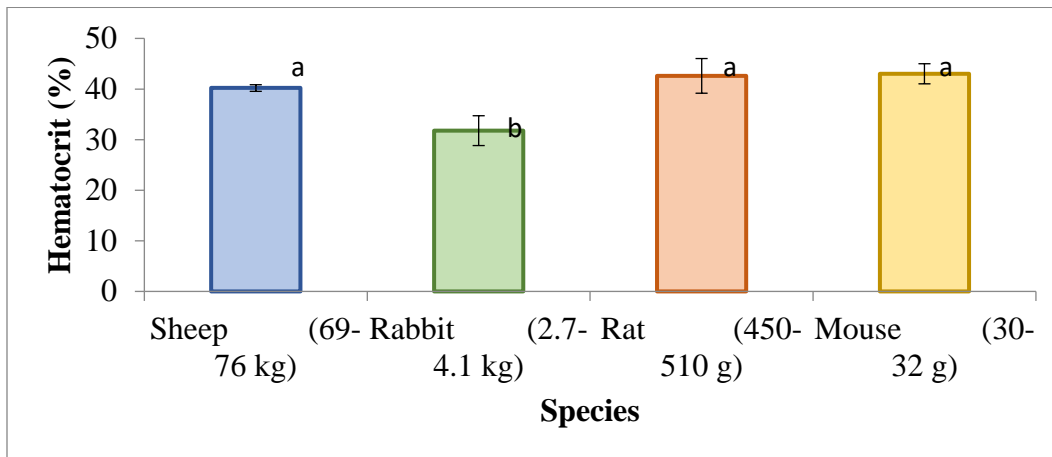
Figure 2: Mean Red Blood Cell Count. For sheep n=3, n=6 for rabbits, n=5 for rats, and n=3 for mice. The error bars represent 95% confidence intervals and letters represent significant differences.

Figure 3: Mean Hematocrit Value of Blood. For sheep n=3, n=6 for rabbits, n=5 for rats, and n=3 for mice. The error bars represent 95% confidence intervals and letters represent significant differences.
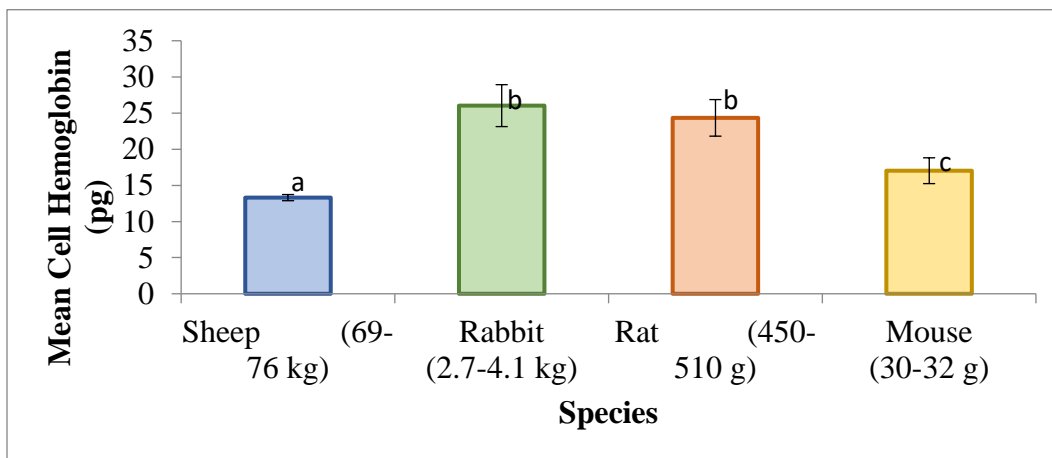

Figure 4: Mean Cell Haemoglobin Concentration of Red Blood Cells. For sheep n=3, n=6 for rabbits, n=5 for rats, and n=3 for mice. The error bars represent 95% confidence intervals and letters represent significant differences.
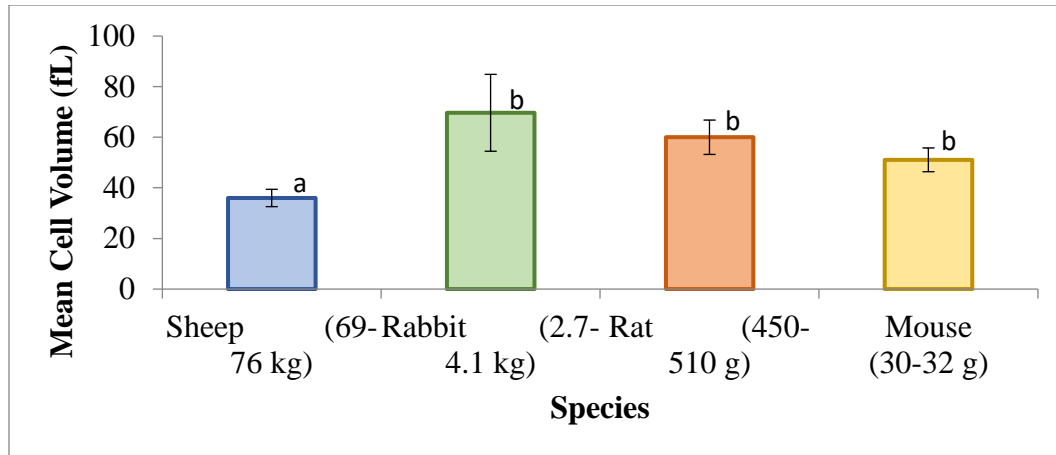
Figure 5: Mean Cell Volume of Red Blood Cells. For sheep n=3, n=6 for rabbits, n=5 rats, and n=3 for mice. The error bars represent 95% confidence intervals and letters represent significant differences.
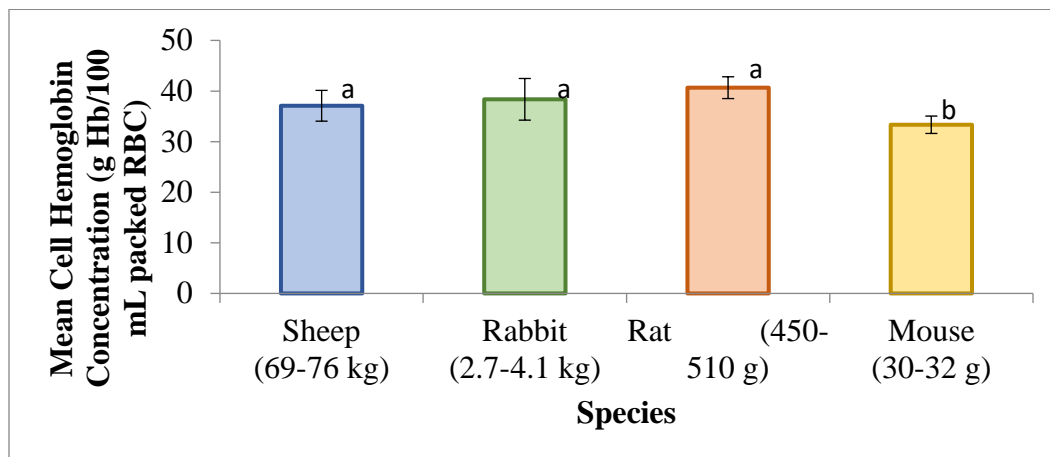

Figure 6: Mean Cell Haemoglobin Content of Red Blood Cells. For sheep n=3, n=6 for rabbits, n=5 for rats, and n=3 for mice. The error bars represent 95% confidence intervals and letters represent significant differences.

Discussion

In this study, we tested the hypothesis that the oxygen carrying capacity of blood is lower in mammalian species with higher body mass. Since the amount of dissolved oxygen in blood is insignificant at physiological partial pressures of oxygen (Pittman 2011 p. 22), the most important contributor to oxygen carrying capacity is the amount of hemoglobin available to bind oxygen. We can assume that the ability of hemoglobin to bind oxygen is similar for all of the species used in this study, since hemoglobin in vertebrates tends to be similar in structure and function (Moyes and Schulte 2016 p. 473), and only vertebrate mammals were selected for this study. Thus, if the blood of species with higher body mass does indeed have decreased oxygen carrying capacity, we expect to see a statistically significant trend where the hemoglobin concentration of whole blood is highest for the mouse, second highest for the rat, second lowest for the rabbit, and lowest for the sheep.

The results we obtained do not correspond with our predictions. We found some significant differences in hemoglobin concentration between species, but these differences do not reveal any consistent effect of increasing body mass on hemoglobin concentration and therefore on oxygen carrying capacity (Figure 1). For example, while we predicted that sheep blood would have the lowest hemoglobin concentration, we found instead that rabbit had the lowest concentration of the four species. We also predicted that mouse blood would have the highest hemoglobin concentration, but found instead that rat had the highest. Overall, the results from this study do not support our proposed hypothesis, since they suggest that body mass between mammalian species has no consistent effect on the oxygen carrying capacity of blood.

As an aside from the main objective of this study, we also investigated whether species body mass had an effect on the quantity of red blood cells or the oxygen carrying characteristics of individual red blood cells. In general, a high fraction or large numbers of red blood cells in the blood indicate an inherently higher oxygen carrying capacity. Additionally, the characteristics of the individual red blood cells can play a substantial role, since the amount of hemoglobin per cell, which is correlated with cell size, affects the amount of oxygen each cell can hold. However, we did not find any clear trends relating body mass to any of our measures of red blood cell abundance (Figure 2) or characteristics of individual red blood cells (Figure 4,5,6).

Our hypothesis that oxygen carrying capacity decreases as body mass increases was not supported. Further research is required, preferably with larger sample sizes, in order to more definitively determine whether there is a consistent effect of body mass on oxygen carrying capacity.

<u>References</u>

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