Relationship between capillaries, mitochondria and maximum power of the heart: a meta-study from shrew to elephant

Heidi D. Horrell[1,2], Anika Lindeque[1], Anthony P. Farrell[3,4], Roger S. Seymour[5], Craig R. White[6], Kayla M. Kruger[1], Edward P. Snelling[1,7]

[1]Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; [2]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; [3]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada; [4]Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia, Canada; [5]School of Biological Sciences, University of Adelaide, Adelaide, South Australia, Australia; [6]Centre for Geometric Biology, School of Biological Sciences, Faculty of Science, Monash University, Clayton, Victoria, Australia; [7]Centre for Veterinary Wildlife Research, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

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ORCID: HDH 0000-0002-0594-3652; AL 0000-0002-7251-4735; RSS 0000-0002-3395-0059; CRW 0000-0002-0200-2187; KMK 0000-0003-1174-8420; EPS 0000-0002-8985-8737

Author for correspondence:
Edward P. Snelling, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, Gauteng, South Africa
Phone: +27 12 529 8245, Email: edward.snelling@up.ac.za

Abstract:

This meta-study uses phylogenetic scaling models across more than 30 species, spanning five orders of magnitude in body mass, to show that cardiac capillary numerical density and mitochondrial volume density decrease with body mass raised to the –0.07 ± 0.03 and –0.04 ± 0.01 exponents, respectively. Thus, while an average 10 g mammal has a cardiac capillary density of approximately 4150 mm^{-2} and a mitochondrial density of 33%, a 1 t mammal has considerably lower corresponding values of 1850 mm^{-2} and 21%. These similar scaling trajectories suggest quantitative matching for the primary oxygen supply and oxygen consuming structures of the heart, supporting economic design at the cellular level of the oxygen cascade in this aerobic organ. These scaling trajectories are nonetheless somewhat shallower than the exponent of –0.11 calculated for the maximum external mechanical power of the cardiac tissue, under conditions of heavy exercise, when oxygen flow between capillaries and mitochondria is likely fully exploited. This mismatch, if substantiated, implies a declining external mechanical efficiency of the heart with increasing body mass, whereby larger individuals put more energy in but get less energy out, a scenario with implications for cardiovascular design, aerobic capacity and limits of body size.

Key words:

Body size, capillaries, heart, mitochondria, power, scaling

Introduction:

The evolution of heart size among mammals of increasing body mass is explained primarily by the large increase in volume loads that act upon the walls of the ventricles in larger species (Snelling et al. 2018), and secondarily by the subtle increase in central arterial blood pressures in larger and taller terrestrial species (Seymour and Blaylock 2000, White and Seymour 2014, Aalkjær and Wang 2021). According to the Principle of Laplace, the increase in volume and pressure loads necessitate an increase in cardiac wall thickness so that wall stress is conserved (Mirsky 1974). While the Laplacian principles that govern variation in heart size as a function of body mass are well understood, the same cannot be said for the factors that drive variation in cardiac ultrastructure. In particular, the relationships between capillary supply and mitochondrial investments of the heart among mammals of varying body mass, and the role of these structures in enabling the vastly different metabolic and mechanical power needed to push blood through the circulatory system, have never been fully teased apart. Analysis of these structures is most relevant because they are responsible for supply of oxygen (capillaries) and consumption of oxygen (mitochondria) in this organ, one that utilises predominantly aerobic metabolic power even during periods requiring sustained maximum external mechanical power, such as heavy exercise (Stanley et al. 2005, Snelling et al. 2016).

Scaling is the traditional approach to understanding how traits are influenced by body mass, whereby a variable (Y) is related to body mass (M_b) by the power equation, $Y = aM_b^{\ b}$, where the coefficient (a) and exponent (b) describe the elevation and shape of the curvilinear line, respectively (Calder III 1996). In recent years, phylogenetic information of species relatedness has been incorporated into these analyses to help satisfy statistical prerequisites pertaining to the independence of data points (Garland and Ives 2000). Using this approach, we recently showed proportionality between capillary supply and mitochondrial investments of the heart across six species of different-sized wild African antelope, evident by the similar scaling exponents derived for total capillary length and for total mitochondrial inner membrane surface area (Snelling et al. 2018). The only previous analysis, across 11 species of mammal, showed that mitochondrial volume density scales with body mass according to a power equation with an exponent significantly less than zero, thus decreasing appreciably from 36.1% in a 2.4 g shrew to 21.1% in a 920 kg cow (Hoppeler et al. 1984). That same

study found that the mitochondrial inner membrane packing density does not change with body mass, based on a subset of three of the species investigated, the wood mouse, cat and cow. To our knowledge, there is no similar analysis on the scaling of capillary supply of the heart. One study, across nine species of mammal, suggested that capillary numerical density likely decreases with increasing body mass (Poupa and Lindström 1983), but the power equation and statistics describing this relationship are unknown.

The capillary and mitochondrial scaling relationships need to be expanded to encompass a larger range of species and body masses, and assessed using modern phylogenetic scaling techniques. Such analyses would allow a formal test for similarity in the scaling exponents that describe the capillary and mitochondrial densities of the heart as a function of body mass, which would indicate proportionality between these two cardiac structures, and lend support to the earlier finding of linear correlation between capillary and mitochondrial densities in the hearts of 13 species of mammal (Hoppeler and Kayar 1988). It holds that a proportional relationship between capillaries and mitochondria would conform to the economic principle of symmorphosis, that structural design at each step of the oxygen cascade is commensurate with the functional capacity of the entire system (Weibel et al. 1991, Weibel et al. 1992, Weibel et al. 1998). This is a testable hypothesis provided we are cognizant that (i) capillary density might be sensitive to fibre cross-sectional area (Egginton and Gaffney 2010), (ii) the relationship could be influenced by secondary functions (e.g., substrate delivery and metabolite removal), and (iii) the relationship could also be influenced by variation in the oxygen carrying capacity of the blood or myoglobin concentration of the surrounding fibres (Hoppeler and Kayar 1988). However, in an aerobic organ like the heart, capillary and mitochondrial densities probably are foremost related to the supply and consumption of oxygen.

If the capillary and mitochondrial structures that support the metabolic power of the heart vary in a similar manner with body mass, then key to understanding this pattern will be knowledge of how the external mechanical power of the heart varies too. Because we assume that the capillaries and mitochondria reflect the maximum metabolic power of the heart during heavy exercise, corresponding to peak rates of blood flow and often accompanied by elevated blood pressure, it is therefore appropriate to consider the sustained maximum external mechanical power of the heart under these

conditions, while understanding that efficiency is such that only a fraction of metabolic power is ultimately converted to external mechanical power to circulate blood around the body (Schipke 1994, Westerhof 2000, Knaapen et al. 2007). The external mechanical power of a normal healthy heart can be approximated as the overall mean rate at which internal pressure energy is imparted to the blood exiting the heart, calculated as the product of cardiac output and mean arterial blood pressure, with sustained maximum levels achieved during heavy exercise (Katz 1932, Blick and Stein 1977). Cardiac output scales with body mass (0.28 – 453 kg) with an exponent of 0.955 ± 0.138 (± 95% confidence interval) in seven mammals undertaking heavy exercise at their aerobic limits, and their heart mass and presumably left ventricular mass scales with an exponent of 1.092 ± 0.123 (data and references in Bishop 1997). Mean arterial blood pressure scales with an exponent of 0.026 (assuming heavy exercise affects the elevation but not the exponent of the blood pressure power equation presented by Calder III 1996). Thus, the maximum external mechanical power of the left ventricular cardiac tissue is hypothesized to scale with an exponent of –0.11 ± 0.05 (0.955 + 0.026 – 1.092). In agreement, a similar exponent is calculated (–0.13 ± 0.20), but with a broader 95% confidence interval due to a narrower body mass range (28.8 – 453 kg), if we applied measured blood pressure recorded in just six of the mammals. A similar exponent would likely be predicted for the right ventricular cardiac tissue too. The kinetic energy of the blood exiting the heart is generally negligible and ignored (Blick and Stein 1977), although the effects of exercise and body mass on kinetic energy are unknown. Therefore, if the capillary numerical density and mitochondrial volume density of the heart also are defined by a similar exponent, then that would suggest matching between the maximum metabolic power and maximum external mechanical power of the cardiac tissue as function of body mass. Conversely, divergence would imply a variable external mechanical efficiency of the cardiac tissue, which might constrain cardiovascular design and aerobic capacity, or even set limits on the body size of mammals.

In this meta-study, we have interrogated the literature for reliable values of cardiac capillary numerical density and mitochondrial volume density for mammalian species of varying body mass. For the first time, we derive phylogenetically informed scaling relationships for the cardiac capillary and mitochondrial densities from over 30 species spanning five orders of magnitude in body mass.

Our aim is to test the hypothesis that similar (parallel) scaling trajectories exist for the capillaries, mitochondria and the maximum external mechanical power of cardiac tissue, indicating proportionality, economy of design, and an external mechanical efficiency that does not change in spite of the enormous variation in heart size from shrew to elephant.

Materials and methods:

The literature was searched for studies on the numerical density of capillaries and volume density of mitochondria in the hearts of mammals, obtained by analysis of light and electron micrographs (Figure 1). Brevity of methodological detail often meant that we could not verify the absolute integrity of the presented values and so most studies were accepted under the assumption that unbiased stereological methods were followed (Howard and Reed 1998, Mühlfeld et al. 2010). The literature search was not exhaustive with priority given to maximising species diversity and body mass range.

Data extracted from the literature included, but were not limited to, the common name, genus, species, sample size, body mass (kg), heart mass (g), relative heart mass (g kg^{-1}), left and right ventricular chamber masses (g), capillary numerical density (profile counts per mm^2 of cardiomyocyte) and mitochondrial volume density (% volume of cardiomyocyte). Units were converted where necessary. Values were accepted only from adult, normal, healthy, control, sham, non-exercised and disease-free groups. Some studies provided paired capillary and mitochondrial densities, although this was not a prerequisite for inclusion. Values were accepted from the myocardium, endocardium, epicardium, and papillary of either the left (free wall and septum) or right ventricular chambers (or both), but never from the atria, and averages were calculated for studies presenting data sampled across multiple regions of the heart. Both immersion and perfusion fixation techniques were accepted. Sometimes heart mass or relative heart mass was calculated provided one of these values was presented together with a value for body mass. Occasionally, body mass was presented as a range, and so the median value was taken. In some instances, studies did not provide any record of body mass, requiring us to estimate based on adult averages for that species (or strain). Similarly, some studies did not identify the species of domestic or laboratory animals, and so the commonly-used species was inserted. In addition, we had to assume data obtained for the agouti belonged to *Dasyprocta leporine*, and data for the elephant was for *Elephas maximus*. Occasionally, mean values were extracted by reverse engineering from figures, performed using the open access web application, WebPlotDigitizer (https://automeris.io/WebPlotDigitizer). Mean species values across studies were then calculated, where appropriate, prior to further analysis.

We used evolutionary relationships to inform our scaling analyses of capillary density, mitochondrial density and heart mass. Mean species values for each of these cardiac traits and for body mass were inspected in the original arithmetic form, prior to $\log_{10}$-transformation and analysis by ordinary least squares (OLS) regression models, and then by phylogenetic generalized least squares (PGLS) regression models (Grafen 1989, Martins and Hansen 1997, Garland and Ives 2000) using the caper v1.0.1 package (Orme 2018) within R v3.6.1 (R Core Team 2017). The evolutionary trees used for the PGLS analyses were constructed from a published supertree of 5020 extant mammals (Fritz et al. 2009). To align our species with those of the constructed trees, it was necessary to combine data for the dog and wolf (*Canis lupus*), the horse and pony (*Equus caballus*), and cattle and steer (*Bos taurus*). A measure of phylogenetic correlation, λ (Pagel 1999), was estimated by fitting PGLS regression models with different values of λ and identifying the value that maximized the log likelihood. We report the PGLS scaling relationships in the form, $Y = aM_b^{\,b \pm 95\%\ CI}$, where Y is the cardiac variable, a is the scaling coefficient (elevation), b is the scaling exponent (slope of the log-transformed relationship), M_b is body mass in kg, and CI is the confidence interval. An F-test determined the statistical significance (set at $P < 0.05$ *a priori*) and the coefficient of determination (r^2) evaluated the strength of the PGLS scaling relationships. The validity of the models were verified by checking for normality and homoscedasticity of residuals. Full details of the PGLS code using the caper package in R, as well as the evolutionary tree, phylogenetic signal, validity of the models, data extraction, species composition, cardiac morphology data and references are available in the supplementary material.

Results:

Sixty-nine published studies of cardiac morphology provided capillary numerical densities for 39 mammal species (~6.94×10^5-fold body mass range) and mitochondrial volume densities for 33 mammal species (~3.94×10^5-fold body mass range). The smallest body mass was a 2.33 g shrew and the largest was a 1.7 t elephant.

Body mass exerted subtle, but significant negative effects on the capillary and mitochondrial densities of the heart, evident by the similar, negative PGLS exponents of $–0.07 \pm 0.03$ ($F_{1,37} = 21.8$, $P < 0.001$) and $–0.04 \pm 0.01$ ($F_{1,31} = 56.0$, $P < 0.001$), respectively (Figure 2). Both exponents are thus statistically different from zero, whereby an average 10 g mammal is predicted to have a cardiac capillary density of approximately 4150 mm^{-2} and a mitochondrial density of 33%, whereas an average 1 t mammal is predicted to have 1850 mm^{-2} and 21%, respectively. In this selection of mammals, heart mass increases in approximate proportion to body mass with a PGLS exponent of 0.96 ± 0.04 ($F_{1,33} = 1950$, $P < 0.001$).

A complete statistical analysis of the PGLS models is provided in Table 1. Validity of the PGLS models, including analysis of the distribution of residuals, q-q plots and density plots, is presented in the supplementary material.

Discussion:

We have used phylogenetically-informed scaling to assess the effect of body mass on the densities of cardiac capillaries and mitochondria of mammals ranging from a 2.33 g shrew to a 1.7 t elephant. Two important findings emerged. First, capillary numerical density and mitochondrial volume density both decrease with body mass following similar (parallel) scaling trajectories, with shallow exponents of –0.07 ± 0.03 and –0.04 ± 0.01, respectively. This suggests quantitative matching of oxygen supply structures and oxygen consuming structures in this aerobic organ. Second, these capillary and mitochondrial densities diverge somewhat from the exponent of –0.11 ± 0.05 calculated for the maximum external mechanical power of the cardiac tissue under conditions of heavy exercise (see Introduction; Calder III 1996, Bishop 1997). A mismatch between the scaling of the capillary and mitochondrial structures supporting maximum metabolic power, and the scaling of maximum external mechanical power, would suggest that the external mechanical efficiency of the cardiac tissue, at least during heavy exercise, declines as a function of body mass.

Parallel scaling of capillaries and mitochondria of the heart

Our exponent of –0.04 ± 0.01 relating cardiac mitochondrial volume density to body mass is identical to that obtained (–0.04) from an earlier assessment of the hearts of 11 species of mammal (Hoppeler et al. 1984). Likewise, our exponent of –0.07 ± 0.03 for cardiac capillary numerical density is very similar to that calculated (–0.06) by extracting and analysing data from a study on the hearts of nine species of mammal (Poupa and Lindström 1983). That our capillary and mitochondrial density exponents are similar indicates parallel scaling of these two traits as a function of body mass across almost the full range for mammals (Figure 2). A parallel scaling between cardiac capillary supply and mitochondrial investments was previously shown across six species of different-sized adult wild African antelope where, relative to the cardiomyocyte reference space, the capillary numerical density matched the mitochondrial inner membrane surface density (Snelling et al. 2018). In this earlier study for a single family of mammals, the negative exponents were somewhat steeper (ca. –0.15) than in the present study assessing general mammalian trends, highlighting that patterns observed within phylogenetic groups do not necessarily translate across all mammals. In general terms, our findings

are also in agreement with the linear correlation between capillary and mitochondrial densities previously seen in the hearts of 13 species of mammal (Hoppeler and Kayar 1988).

A better appreciation of our finding of consistently similar cardiac capillary and mitochondrial scaling exponents, which suggest a proportionality between these two terminal structures of the oxygen cascade, requires consideration of their crucial roles within the heart. Capillary numerical density is approximately equivalent to its length density due to its modest tortuosity in cardiac tissue, and so can be considered a morphometric descriptor of local oxygen supply capacity (Hoppeler et al. 1981b, Hoppeler and Kayar 1988), especially when applied to aerobic cardiac tissue where secondary functions such as substrate delivery, lactate and CO_2 removal, and heat dispersal are assumed to have less influence on design. In a similar way, mitochondrial volume density can be viewed as a morphometric descriptor of local oxygen consumption capacity (Hoppeler et al. 1981a, Hoppeler and Kayar 1988), because the packing density of the mitochondria's inner membrane, with its embedded enzymes for oxidative phosphorylation, likely does not vary across a broad spectrum of body mass (Hoppeler et al. 1984). Thus, the parallel scaling of the quantitative characteristics of a structure responsible for the supply of oxygen (capillaries) and those of a structure that ultimately uses that oxygen (mitochondria) provides direct support for economic design at the cellular level of the oxygen cascade in an aerobic organ. Indeed, it is indicative of the principle of symmorphosis, that no more structure should exist in a system, including the steps of the oxygen cascade, than is necessary to satisfy the functional capacity of the system, assuming the driving design feature is the transport and consumption of oxygen.

Mismatch of capillaries and mitochondria with the mechanical power of the heart

While the scaling exponents for cardiac capillary and mitochondrial densities are similar (–0.07 ± 0.03 and –0.04 ± 0.01, respectively), the exponent for the maximum external mechanical power of the cardiac tissue, under conditions of heavy exercise, has rather a more negative scaling exponent of –0.11 ± 0.05. Although the differences appear modest, if substantiated when more data become available, they imply that the external mechanical efficiency of the cardiac tissue, defined as the ratio of external mechanical power and metabolic power, decreases with body mass raised to an exponent

of approximately –0.055 (–0.11 – [average of –0.07 and –0.04]), having a potentially significant effect when considered across the full body mass range of mammals. For instance, if we were to take an external mechanical efficiency of 25% for a 60 kg human undertaking heavy exercise (Krasnow et al. 1964, Gibbs 1978, Schipke 1994), an exponent of –0.055 would predict a decline in efficiency from 40% in a 10 g mammal to 21% in a 1 t mammal. This would contradict the traditional view that rapidly beating smaller hearts are less efficient than slowly beating larger hearts (Krasnow et al. 1964); in fact, if anything, our results suggest that it is the larger hearts that are less efficient than the smaller ones, at least under conditions of heavy exercise when cardiac tissue is required to perform at its sustainable limits.

A declining external mechanical efficiency of the heart with increasing body mass implies that a disproportionate amount of metabolic energy is not converted to internal pressure energy of blood exiting the heart (i.e., cardiac output × mean arterial blood pressure) in larger mammals. Potentially, this might arise if the kinetic energy of the blood increases disproportionately with body mass, from being a trivial component of total external mechanical power in smaller mammals to becoming a more important contributor in larger mammals. It has been suggested that blood velocity, and thus its relative kinetic energy, could increase with body mass (Calder III 1996), but this has never been confirmed empirically, and it is generally considered to represent only a small fraction of the total external mechanical power of a normal healthy heart. The apparent declining external mechanical efficiency of the heart with increasing body mass might also be explained if a disproportionate amount of energy never exits the system and instead is lost internally as heat. This might occur if the relative efficiency of certain energy conversion processes of the cardiac tissue decrease in larger mammals, such as those involved in the synthesis of ATP at the mitochondria, and those involved in the hydrolysis of ATP at the myofibril cross-bridges or at the various cellular ion pumps. It is further possible that a disproportionate amount of energy might also be lost if relatively more work is done to deform the relatively thicker ventricular walls of larger mammals. Whatever the sources of energy loss in the system, it is worth contemplating that while it remains a reasonable evolutionary hypothesis that selection may optimise efficiency under routine cardiac workloads that mammals experience most of the time, it also is reasonable to suspect that selection may be geared

towards maximising the power output of the heart, at the expense of efficiency, under the occasional periods of heavy exercise (and subsequent recovery) when survival is threatened. There is some evidence to suggest that workloads that optimise efficiency are different from those that optimise power output (Toorop et al. 1988).

Conclusion

This meta-study shows that both the capillary numerical density and mitochondrial volume density of the mammalian heart decreases with body mass with parallel scaling trajectories. This finding is in accordance with limited previous data, and so we propose mammalian terminal cardiac oxygen supply structures and oxygen consuming structures are quantitatively matched, providing support for the economic design principles of symmorphosis at the cellular level of the oxygen cascade in an aerobic organ. However, it was also shown that the scaling of the cardiac capillary and mitochondrial densities are rather out of step with that of the calculated maximum external mechanical power of the cardiac tissue, which if substantiated, would indicate a decreasing external mechanical efficiency of the heart with increasing body mass, at least under conditions of heavy exercise. If the efficiency of the heart, a critical life support organ, decreases with body mass, then it would imply that larger mammals, compared to their smaller counterparts, expend a disproportionate amount of metabolic energy in the cardiac tissue relative to the amount of mechanical energy imparted to the blood. This would have implications for the evolution of cardiovascular design and aerobic capacity, and it may even influence the limits of body size among mammals.

Abbreviations:

M_b, body mass; OLS, ordinary least squares; PGLS, phylogenetic generalized least squares.

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Author contributions:

EPS, original concept; HH, AL, KMK, EPS, data collection; CRW, EPS, data analysis; HH, AL, APF, RSS, CRW, KMK, EPS, data interpretation; HH, EPS, wrote the paper; HH, AL, APF, RSS, CRW, KMK, EPS, edited and approved the paper.

Conflict of interest:

No conflicts of interest, financial or otherwise, are declared by the authors.

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Data availability:

Data available in supplementary material and on ResearchGate (www.researchgate.net/profile/Edward_Snelling).

Figure captions:

Figure 1: The close functional relationship between the capillaries that supply oxygen (17 large tubular profiles flushed of blood) and mitochondria that consume oxygen (numerous dark-stained organelles within the surrounding cardiomyocytes) is evident by their close morphological association as shown in this electron micrograph captured from the heart of a wild African antelope, the common duiker *Sylvicapra grimmia*.

Figure 2: The relative investment of capillaries and mitochondria in the heart decreases in a proportional manner as body mass increases across five orders of magnitude among mammals. According to the PGLS analyses, (A) capillary numerical density (profile counts per mm^2 of cardiomyocyte) across 39 species decreases with body mass raised to the exponent of $–0.07 \pm 0.03$ ($r^2 = 0.37$, $F_{1,37} = 21.8$, $P < 0.001$), and (B) mitochondrial volume density (% volume of cardiomyocyte) across 33 species decreases with an exponent of $–0.04 \pm 0.01$ ($r^2 = 0.64$, $F_{1,31} = 56.0$, $P < 0.001$). Both exponents are statistically different from zero.

Tables:

Table 1: Scaling relationships and statistics for capillary numerical density (profile counts per mm^2 of cardiomyocyte), mitochondrial volume density (% volume of cardiomyocyte) and heart mass (g) expressed as a function of body mass (M_b; kg) according to PGLS and OLS analyses.

	Capillary density (mm^{-2})	Mitochondrial density (%)	Heart mass (g)
PGLS	$2997M_b^{-0.07 \pm 0.03}$ $r^2 = 0.37$, $F_{1,37} = 21.8$, $P < 0.001$, $\lambda = 0.672$	$27.4M_b^{-0.04 \pm 0.01}$ $r^2 = 0.64$, $F_{1,31} = 56.0$, $P < 0.001$, $\lambda = 0.530$	$6.09M_b^{0.96 \pm 0.04}$ $r^2 = 0.98$, $F_{1,33} = 1950$, $P < 0.001$, $\lambda = 0.443$
OLS	$3464M_b^{-0.05 \pm 0.03}$ $r^2 = 0.34$, $F_{1,37} = 18.8$, $P < 0.001$	$27.3M_b^{-0.05 \pm 0.01}$ $r^2 = 0.77$, $F_{1,31} = 102.4$, $P < 0.001$	$5.82M_b^{0.97 \pm 0.04}$ $r^2 = 0.99$, $F_{1,33} = 2696$, $P < 0.001$
N = values	80	92	76
N = species	39	33	35
Body mass range	2.45 g to 1.70 t	2.33 g to 920 kg	2.40 g to 920 kg

Equations are in the form $Y = aM_b^{b \pm 95\% CI}$, where Y is the cardiac trait, a is the scaling coefficient (elevation) and b is the scaling exponent (slope of the log-transformed relationship). The F-test and accompanying P value assess the statistical significance of the relationships, r^2 is the coefficient of determination to evaluate their strength, and λ is the measure of phylogenetic correlation. The validity of the PGLS models, verified by checking for normality and homoscedasticity of residuals, is presented in the supplementary material. ANCOVA of the OLS relationships for capillary and mitochondrial densities against body mass confirm no statistical difference among slopes, i.e., the respective exponents are similar ($F_{1,68} = 0.19$, $P = 0.67$).

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