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Heart design: free ADP scales with absolute mitochondrial and myofibrillar volumes from mouse to human

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Abstract

Our aim was to estimate a number of bioenergetic parameters in the beating mouse, rat and guinea pig heart in situ and compare the values to those in hearts of mammals over a 2000-fold range in body mass. For the mouse, rat and guinea pig heart, we report a phosphorylation ratio of 1005 ± 50 ($n = 16$), 460 ± 32 ($n = 10$) and 330 ± 22 ($n = 5$) mM^{-1} and a free cytosolic [ADP] concentration of 13, 18 and 22 μM, respectively. When each parameter was plotted against body mass, they scaled closely to the quarter power (-0.28, $r = 0.99$ and -0.23, $r = 0.97$). A similar regression slope was found when the inverse of free [ADP] was plotted against absolute mitochondrial (slope = -0.26, $r = 0.99$) and myofibrillar volumes (slope = -0.24, $r = 0.99$). The similar slopes indicate that the ratio of absolute mitochondria and myofibrillar volumes in the healthy mammalian heart is a constant, and independent of body size. In conclusion, our study supports the hypothesis that the mammalian heart has a number of highly conserved thermodynamic and kinetic parameters that obey quarter-power laws linking the phosphorylation ratio, ATP turnover rates, free [ADP] and absolute mitochondrial volumes to body size. The results are discussed in terms of possible mechanisms and potential deviations from these laws in some disease states.

Keywords: Heart; Adenosine 5′-diphosphate; Mitochondria; Scaling; Oxygen consumption

1. Introduction

The energy cost of living is more expensive if you are small. One gram of mouse heart, for example, consumes 7–10 times more oxygen (and therefore ATP) than 1 g of human tissue [2]. Smaller mammals per unit body mass consume more oxygen, eat more food, produce more heat and live shorter lives compared to larger ones [1–5]. When plotted on logarithmic coordinates the mass-specific oxygen consumption scales inversely with body size with a regression slope of a quarter power or 0.25 [2,6].

$$V_{O_2}/P_{met} = 0.676\ M_b^{-0.25} \quad (1)$$

where V_{O_2}/P_{met} is the mass-specific oxygen consumption in liters O_2 per kilogram body mass per hour [2]. Higher mass-specific oxygen consumption rates have been associated with increased maximal enzyme activities of key glycolytic and Krebs cycle enzymes

Abbreviations: PCr, phosphocreatine; Cr, creatine; CK, creatine kinase; P_i, inorganic phosphate; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; free [Mg^{2+}], unbound magnesium; ^{31}P-NMR, phosphorus nuclear magnetic resonance; V_{mit} and V_{myofib}, total volume of mitochondria and myofibrils, respectively; V_{O_2}, volume of oxygen consumed by the body

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[5,7,8], increased cytochrome oxidase activities and contents [9,10], as well as absolute mitochondrial volumes [11–18]. Other physiological variables such as heart rate, respiratory rate, tissue blood flow and food intake have also been shown to scale which led West and colleagues to propose a general model for the origin of the scaling relationships based on the transport of materials through linear networks that branch to supply all parts of the organism [19].

In heart, the scaling of two metabolic parameters that have received little attention are free cytosolic [ADP] and the cytosolic phosphorylation ratio ([ATP]/[ADP][P_i]) and their relationships to absolute mitochondrial and myofibrillar volumes [20]. Free [ADP] is important because of its possible role in the regulation of oxygen consumption in heart and skeletal muscle [21] and [ATP]/[ADP][P_i] for providing the cellular driving potential between ATP and its products ADP and P_i. The [ATP]/[ADP][P_i] is linked to the transformed Gibbs energy through $\Delta G'_{ATP} = \Delta G'^{\circ}_{ATP} + RT \ln([ADP][P_i]/[ATP])$ [22,23]. Thus, the first aim of this study was to test our scaling hypothesis in hearts of smaller mammals using a purpose built ^{31}P-NMR surface coil and a DEPTH pulse sequence was chosen to reduce contamination from chamber blood. The second aim was to establish the relationship between free cytosolic [ADP] (and the [ATP]/[ADP][P_i] ratio) and absolute mitochondrial and myofibrillar volumes over a 2000-fold difference in heart mass and body size.

2. Materials and methods

2.1. In situ heart preparation

Healthy, mature Quacker-bush mice (32 ± 2 g), Sprague–Dawley rats (300 ± 16 g) and guinea pigs (850 ± 45 g) were anesthetized with Nembutal (~60 mg/kg body wt.) (Boehringer Ingelheim). A tracheotomy was performed and animals were artificially ventilated with a Harvard small animal ventilator. The displacement volumes and rates were: mouse (1.5 ml, 165 min^{-1}), rat (3 ml, 60–70 min^{-1}) and guinea pig (4–4.5 ml, 45 min^{-1}). The heart was exposed by a thoracotomy and the animal placed in a temperature-controlled animal cradle (38°C). A purpose built ^{31}P-NMR surface coil with a flexible arm was positioned against the left ventricular myocardium [20,24]. The cradle was introduced into the horizontal bore magnet about 20 min after the commencement of surgery and remained in the magnet for the duration of the experiment (usually 1–2 h). A separate group of animals (12 mice, three rats and three guinea pigs) were anesthetized and instrumented for the biochemical determination of the total creatine (Cr) concentration (see below). Heart tissue was freeze-clamped in tongs cooled in liquid N_2. Frozen wafers were stored at −80°C until analyzed.

2.2. NMR experiments

^{31}P-NMR spectra were recorded at 121.452 MHz with a 11 cm horizontal bore, 7.05 Tesla magnet connected to a Varian INOVA console. A three-turn coil with an inner diameter of 3.5 mm was built from enameled copper wire (W3124, Versa Electronics, Australia) of 0.5 mm thickness. The coil was tuned and matched with ceramic capacitors of variable capacity to resonate at 121.45 MHz. The Q-factor of the coil was 102 in air (unloaded) and 47 when loaded. The signal was maximized by shimming the ^{31}P-NMR surface coil off-resonance on the myocardial ^{1}H-NMR signal. A DEPTH pulse sequence (180°–90°–180°–180°) [25,26] was used to obtain 512 signal-averaged FIDs. A relaxation delay of 2 s was applied. Calibration factors compensating for partial saturation were determined by comparing spectra using the 2 s relaxation delay with fully relaxed spectra with a relaxation delay of $5 \times T_1$ (15 s for the metabolites in the hearts in situ and 60 s for the phenylphosphonate in the phantom, respectively) [24]. A coil sensitive depth profile was determined with a phantom consisting of four layers of a known thickness (between 0.8 and 1.1 mm) and containing 10 mM of different phosphorylated compounds. From this procedure it was possible to determine the excitation band of the coil. Metabolite concentrations in the ^{31}P-NMR spectra were quantified by comparison of the respective signal areas from the tissue to an external standard containing 10 mM phenylphosphonite that was placed directly on top of the surface coil in exactly the same geometrical arrangement. We believe that this method provides similar molar sensitivities in the standard and tissue as shown by Hitchins et al. [27].

2.3. Calculation of phosphorus metabolites, [ATP]/[ADP][P_i] ratio and free [ADP]

The concentration of ATP, phosphocreatine (PCr) and P_i were determined from the integrals of the in situ ^{31}P-NMR spectrum using the DEPTH pulse sequence relative to phosphorus signal in the external standard. To calculate ATP concentrations from the NMR spectrum ATP was assumed to equal 93% of the β-NTP (nucleoside triphosphate) intensity [28]. The intracellular space of 73% of the total tissue water was used for calculation of cell concentrations where TTW = 0.79 ml g wet wt.$^{-1}$ [29,32].

Free cytosolic [ADP] and [ATP]/[ADP][P_i] were calculated by substituting the metabolic data (in moles/l cell water) into the creatine kinase equilibrium after adjusting the creatine kinase (CK) constant to the pH and free [Mg^{2+}] of the heart [23]. Total creatine was determined separately on freeze-clamped hearts by measuring PCr and Cr enzymatically using fluorometric procedures [30]. Total creatine (PCr+Cr) was measured enzymatically on freeze-clamped tissue and found to be 17 ± 0.71 μmoles ml^{-1} intracellular water (n = 8, S.E.M.), and intracellular Cr concentration calculated from subtracting the NMR derived PCr from the total Cr concentration. Cytosolic free [Mg^{2+}] was estimated from the chemical shift difference of the α- and β-resonances of ATP [31] and pH was determined either from the chemical shift of the P_i signal relative to the PCr signal or alternatively from the chemical shift difference of the β- and γ-resonances of ATP [32].

2.4. Estimation of cardiac output of different mammals and absolute mitochondrial and myofibrillar volumes

The cardiac outputs of the mouse, human and elephant were calculated from the relation $Q_h = 187\ M_b^{0.81}$ where Q_h is the cardiac output (ml min^{-1}) and M_b is body mass in kg [2]. The cardiac output for the 30 g mouse was 10 ml min^{-1} (heart rate 600 beats min^{-1}), for the 70 kg human 5 l min^{-1} (heart rate 70 beats min^{-1}), and for the 3800 kg elephant 148 l min^{-1} (heart rate 30 beats min^{-1}). The heart weight of the mouse was 0.15 g, the human heart was 400 g and the elephant heart 19 kg. The life span of a mouse is 3 years, of a human 70 years and of an elephant 50 years. Similar calculations have shown the same for the number of heart beats over a life time (about 1500 million heartbeats) but notable exceptions exist [1]. Absolute mitochondrial volumes in hearts of different species were obtained from volume densities reported by Kim et al. [18], Schaper et al. [13] and Hoppler et al. [12]. The absolute volume of heart mitochondria (V_{mit}) was obtained by multiplying the volume densities with heart mass and dividing by the density of muscle tissue (1.06 g cm^{-3}) [12].

2.5. Statistics

Unless stated otherwise, all values shown are means ± S.E.M. with the number of samples in parentheses. Linear regression analysis was used to determine the relationship between the bioenergetic parameters and body or heart mass, or absolute mitochondrial and myofibrillar volumes. The error bars represent the standard error of the mean. The level of statistical significance was $P < 0.05$.

3. Results

Representative ^{31}P-NMR spectra of the guinea pig, rat and mouse heart are shown in Fig. 1a, b and c respectively. The DEPTH pulse sequence (180°–90°–180°–180°) did not exceed 1 mm (approximate thickness of the left ventricle of the mouse) and was verified using a multi-layer phantom (data not shown). A comparison with data acquired with a 30° pulse sequence revealed a marked suppression of contaminating phosphate at the inorganic phosphate resonance that we assume was 2,3-diphosphoglycerate in the vascular and ventricular blood.

The ^{31}P-NMR determined metabolites and bioenergetic parameters in hearts of three mammalian species are reported in Table 1. The [PCr]/[ATP] ratios in situ were 2.70 ± 0.08 (S.E.M., n = 16), 2.40 ± 0.03 (n = 10) and 2.30 ± 0.04 (n = 5) in mouse, rat and guinea pig heart, respectively, for a cytosolic free [ADP] of 12.9, 17.5 and 21.8 μM (Table 1). The phosphorylation ([ATP]/[ADP][P_i]) ratios in the mouse, rat and guinea pig heart in vivo were 1005, 460 and 330 mM^{-1} for a $\Delta G'_{ATP}$ of −70, −67.5 and −66.5 kJ mol^{-1} (Table 1). These ratios for the

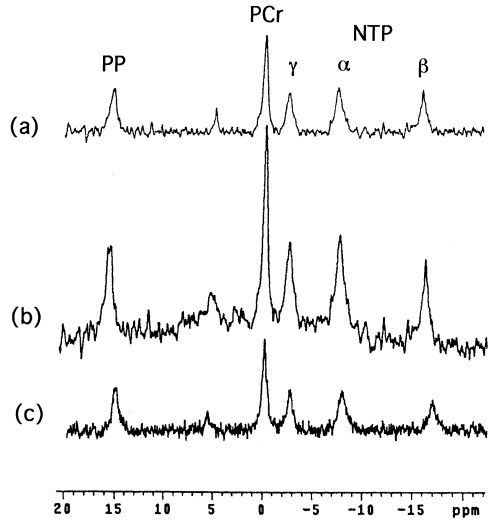

Fig. 1. ^{31}P-NMR spectra of the resting (non-paced) in situ heart ventricle of guinea pig (a), rat (b) and mouse (c). Spectra were acquired with a DEPTH pulse sequence (180°–90°–180°–180°–acq.). A total of 512 signal-averaged FIDs were acquired (total NMR time: 17 min). Absolute concentrations of phosphorylated metabolites in the myocardium were calculated by comparison with an external standard (10 mM phenylphosphonite, PP) that was placed on top of the coil in a similar geometric arrangement compared to the heart (see Section 2).

mouse, rat and guinea pig are also in good agreement with values predicted from the relationship $[ATP]/[ADP][P_i]$ (mM) = 386.4 $M_b^{-0.30}$ [20]. Fig. 2a shows the relationship between the myocardial $[ATP]/[ADP][P_i]$ and heart mass which scales with a regression slope −0.28 ($r = 0.99$). The equation describing the relationship between the heart cytosolic phosphorylation ratio and mass-specific tissue oxygen consumption is $[ATP]/[ADP][P_i] = (V_{O_2}/P_{met})^{1.18}$ ($r = 0.99$) with $[ATP]/[ADP][P_i]$ in mM^{-1} and V_{O_2}/P_{met} in liters O_2 h^{-1} kg^{-1} (graph not shown). Fig. 2b shows a similar relationship and slope between the inverse of free cytosolic [ADP] and heart mass (regression slope = −0.23, $r = 0.98$). The similar slopes between Fig. 2a and b demonstrate that the major factor maintaining the higher phosphorylation ratio in smaller mammalian hearts is the maintenance of lower micromolar concentrations of free cytosolic [ADP].

Fig. 2c shows the relationship between the inverse of free myocardial cytosolic [ADP] and absolute mitochondrial and myofibrillar volumes (cm^3). The regression slope describing free [ADP] and mitochondrial volume was −0.26 ($r = 0.99$) and free [ADP] and myofibrillar volume was −0.24 ($r = 0.98$), and close to a quarter power.

4. Discussion

Our study demonstrates a number of findings: first, the heart cytosolic phosphorylation ratio (ATP/ADP P_i) and the inverse of free [ADP] both scale with body mass with a slope close to a quarter power (Fig. 2a,b), and second, the inverse of free [ADP] also scales with absolute volumes of mitochondrial (V_{mit}) or myofibrillar volumes (V_{myofib}) with similar quarter-power slopes (Fig. 2c). It is proposed that these mass-specific quarter-power allometric relations have important implications in understanding cardiac function in health and disease (see below).

Table 1
^{31}P-NMR determined metabolites and bioenergetic parameters in mouse, rat and guinea pig hearts in situ

Species	n	PCr	ATP	Free Mg^{2+}	pH_i	Free ADP	P_i	ATP/ADP P_i	$\Delta G'_{ATP}$
Mouse (32 g)	22	13.8 ± 0.3	4.9 ± 0.1	0.41 ± 0.02	7.32 ± 0.02	12.9 ± 0.3	< 0.4	1005 ± 43	−69.9
Rat (300 g)	10	12.2 ± 0.31	5.4 ± 0.09	0.45 ± 0.08	7.27 ± 0.03	17.5 ± 0.6	0.79 ± 0.03	460 ± 32	−67.5
Guinea pig (850 g)	5	11.4 ± 0.45	5.6 ± 0.13	0.48 ± 0.02	7.25 ± 0.02	21.8 ± 0.9	0.98 ± 0.04	330 ± 22	−66.5

Values are expressed in mM with the exception of free ADP (μM) and $\Delta G'_{ATP}$ (kJ mol^{-1}). Creatine [Cr] was calculated by subtracting the NMR derived PCr from the total Cr [TCr] determined by biochemical assay where the [TCr] for mouse, rat and guinea pig heart was 18.8 ± 0.43 ($n = 12$), 23.7 ± 0.3 ($n = 3$) and 25.9 ± 0.5 ($n = 3$). Conversion of total tissue content (μmol/g wet wt.) to intracellular concentrations (mM) was performed using intracellular space of 73% and total tissue water of 0.79 g H_2O g^{-1} wet tissue determined in heart (see text). Values are mean ± S.E.

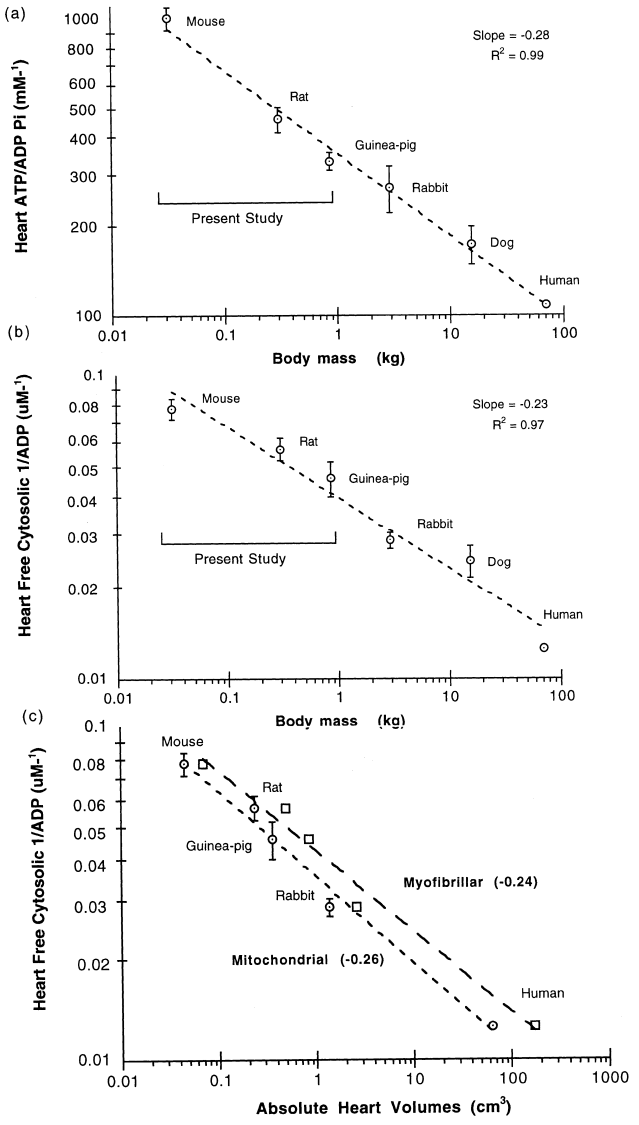

Fig. 2. Correlation between cardiac bioenergetic parameters, mass and mitochondrial and myofibrillar volumes from mouse to human. (a) Cytosolic phosphorylation ratio as function of mammalian heart mass (M_b, kg). The equation to the line is $[ATP]/[ADP][P_i]$ (mM) = 559.86 $M_b^{-0.28}$ ($r = 0.99$). (b) Cytosolic free [ADP] (μM) as a function of heart mass (M_b, kg) is $[1/ADP] = 0.0583\ M_b^{-0.23}$ ($r = 0.97$). (c) Cytosolic free [ADP] (μM) as a function of absolute mitochondrial and myofibrillar volumes (cm^3). For mitochondria (□) V_{mit} the equation to the line is $[1/ADP] = 0.0416\ (V_{mit})^{-0.26}$ ($r = 0.99$). For myofibrillar volumes (○), V_{myofib}, the equation to the line is $[1/ADP] = 0.0492\ (V_{myofib})^{-0.24}$ ($r = 0.98$). Absolute mitochondrial volumes in hearts of different species were obtained from Kim et al. [18], Schaper et al. [13] and Hoppler et al. [12].

4.1. Scaling of the myocardial phosphorylation ratio and free [ADP]

When the heart ATP/ADP P_i ratio or inverse of free [ADP] is plotted as a function of body mass (Table 1, Fig. 2a,b), the regression lines have slopes of −0.28 ($r = 0.99$) and −0.23 ($r = 0.97$), respectively, and support the bioenergetic scaling hypothesis of Dobson and Headrick [20]. The higher phosphorylation ratio, higher $\Delta G'_{ATP}$, and higher mass-specific metabolic rate in the mouse heart is consistent with the scaling of a number of key glycolytic and mitochondrial enzymes in smaller mammals [5,7,8], including a higher capacity for Ca^{2+} re-uptake by the sarcoplasmic reticulum required with higher myosin ATPase activities [33]. However, it is not known whether the higher myocardial phosphorylation ratios (and lower free [ADP]) in the mouse heart are maintained at higher mitochondrial proton motive forces and/or higher $NADH/NAD^+$ ratios than in hearts of larger species.

4.2. Scaling of absolute mitochondrial and myofibrillar volumes

The second major finding of the present study was that the inverse of free [ADP] from mouse to human also scaled close to the quarter power with absolute volumes of mitochondrial (V_{mit}) and myofibrillar volumes (V_{myofib}). To our knowledge, this is the first time free [ADP] and absolute mitochondrial and myofibrillar volumes have been shown to scale with body mass (and heart mass) in mammals. Previous work on mitochondrial scaling in heart has concentrated on the relation to body mass, and maximal whole body oxygen consumption. In 1984, Hoppeler and colleagues found that the absolute volume of mitochondria in heart from shrew to cow also scaled against body mass with a slope of 0.93 [12].

Our observation that free [ADP] is lower as the absolute mitochondrial (and myofibrillar) volume increases with decreasing heart (and body) size is significant given that free [ADP] has been reported to be a potential regulator of oxygen consumption [21,34]. Not only do increased absolute mitochondrial volumes lead to increased ATP turnover rates in the hearts of smaller mammals, but the lower steady-state concentration of [ADP] may provide

greater gain in controlling the rate of oxygen consumption. The low concentration of free [ADP] is similar to the apparent K_m value of oxidative phosphorylation in isolated mitochondria (20–30 μM) [35]. However, it should be emphasized that correlations between free [ADP] and cardiac oxygen consumption do not imply causality, and further studies are required to settle this issue.

Another important finding of the present study was the observation that the ratio of absolute myofibrillar to mitochondrial volume in heart is a constant, and independent of body size in mammals. From the similar slope of regression lines in Fig. 2c, the ratio was calculated to be around 2–1 – or the absolute myofibrillar volume is 2 times the absolute mitochondrial volume in the mammalian heart. The relation expressed mathematically is $V_{mit} = 0.52 (V_{myofib})^{0.93}$ ($r = 0.99$) where V_{mit} is the absolute mitochondrial and V_{myofib} the absolute myofibrillar volume (graph not shown). Maintaining this precise ratio in heart appears to be essential in coupling ATP utilization to ATP synthesis.

Disruption of this ratio of absolute myofibrillar to mitochondrial volume is likely to be associated with morphological, cellular, metabolic and bioenergetic dysfunction such as in cardiac hypertrophy, a significant risk factor for congestive heart failure. Ultrastructural studies of Breisch et al. in cats have shown that there is indeed a mismatch in the myofibrillar to mitochondrial ratio at 4 and 7 days of pressure overload hypertrophy, which then returns to normal after 30 days [36]. However, in later stages (120–148 days) an increase in the absolute mitochondrial volume relative to the myofibrillar volume occurs along with a continual decrease in capillary density and reduced cardiac function [36]. More recently, the importance of maintaining a fixed ratio of 2 between absolute volume of myofibrils and mitochondria was shown in the study of Knaapen and colleagues during the development of embryonic rat myocardium (11–17 days) [37].

4.3. Significance of findings and perspective

The question of central importance in this study is what is the significance of a mass-specific quarter-power allometry that is so pervasive in mammals, and other multicellular organisms. This question has entertained physiologists for over 100 years since the original observations of Rubner [2]. The most recent attempt to explain the mass-specific quarter-power law is by West and colleagues who argue that multicellular organisms have evolved hierarchical branching networks that terminate in size-invariant units such as capillaries and mitochondria [38]. Natural selection has tended to maximize both metabolic capacity and internal efficiency; the former by maximizing the scaling of exchange surface areas, and the latter by minimizing the scaling of transport distances and times [38]. Thus, according to this model, the scaling of metabolic rate and bioenergetic parameters in heart, and their precise relations to absolute mitochondria and myofibrillar volumes, appear to be the result of evolutionary selection to maximize the capacities to deliver and exchange oxygen and oxidizable fuels and remove waste products over optimal transport distances and times. These mass-specific quarter-power laws in heart, and the design constraints they underpin, appear to be highly conserved phenomena. To give an example of how the scaling of structural, metabolic and bioenergetic parameters lead to highly conserved features in the mammalian heart design, the tiny mouse heart weighing 0.15 g and beating at 600 beats min^{-1} will pump similar blood volumes *on a mass basis* over a 3 year life span as the 0.4 kg human heart beating at 70 beats min^{-1} over a 70 year life span, or the 19 kg elephant heart beating at 30 beats min^{-1} over a 50 year life span. The volume of blood pumped per kg for the mammalian heart over its respective life span is around 300 000 000 l kg $heart^{-1}$ $lifetime^{-1}$.

4.4. Conclusion

We conclude that the abilities of the mouse heart to pump faster than the human heart over a 2000-fold difference in body mass is a direct consequence of negative quarter-power allometric laws built into its structural, metabolic and bioenergetic design. Our scaling data indicate that there is a natural selection of 'fitness' per gram heart where mass-specific (negative) quarter-power scaling is a highly conserved consequence of supplying a whole animal with linear networks to meet a (positive) three-quarter-power scaled whole organism.

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