Biphasic Allometry of Cardiac Growth in the Developing Kangaroo *Macropus fuliginosus**

Edward P. Snelling^{1,†}

David A. Taggart¹

Shane K. Maloney²

Anthony P. Farrell³

Roger S. Seymour¹

¹School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia; ²School of Anatomy, Physiology and Human Biology, University of Western Australia, Crawley, Western Australia 6009, Australia; ³Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada; and Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

Accepted 11/21/2014; Electronically Published 1/8/2015

ABSTRACT

Interspecific studies of adult mammals show that heart mass (M_h , g) increases in direct proportion to body mass (M_b , kg), such that $M_h \propto M_b^{1.00}$. However, intraspecific studies on heart mass in mammals at different stages of development reveal considerable variation between species, $M_h \propto M_b^{0.70-1.00}$. Part of this variation may arise as a result of the narrow body size range of growing placental mammals, from birth to adulthood. Marsupial mammals are born relatively small and offer an opportunity to examine the ontogeny of heart mass over a much broader body size range. Data from 29 western grey kangaroos *Macropus fuliginosus* spanning 800-fold in body mass (0.084–67.5 kg) reveal the exponent for heart mass decreases significantly when the joey leaves the pouch (ca. 5–6 kg body mass). In the pouch, the heart mass of joeys scales with hyperallometry, $M_{h(\text{in-pouch})} = 6.39M_b^{1.10 \pm 0.05}$, whereas in free-roaming juveniles and adults, heart mass scales with hypoallometry, $M_{h(\text{postpouch})} = 14.2M_b^{0.77 \pm 0.08}$. Measurements of heart height, width, and depth support this finding. The relatively steep heart growth allometry during in-pouch development is consistent with the increase in relative cardiac demands as joeys develop endothermy and the capacity for hopping locomotion.

Once out of the pouch, the exponent decreases sharply, possibly because the energy required for hopping is independent of speed, and the efficiency of energy storage during hopping increases as the kangaroo grows. The right : left ventricular mass ratios (0.30–0.35) do not change over the body mass range and are similar to those of other mammals, reflecting the principle of Laplace for the heart.

Introduction

The principle of Laplace explains that as pressure loading and volume loading on the heart increase, so too must the thickness of the ventricular wall, ensuring that wall stress (N m^{-2}) is conserved and the load is spread (Grande and Taylor 1965). The mammalian heart operates under a considerable range of pressure and volume loads, much of which can be explained by the wide body mass range of mammals. Volume loading increases with body mass to account for the increasing requirements for cardiac output, while pressure loading increases as larger, and particularly taller, terrestrial mammals support a higher vertical column of blood above the heart (Seymour and Blaylock 2000). Some of the variation in loading also exists independent of body mass. Large volume loads occur, for example, in mammals that are particularly athletic or in response to training (Ford 1976), while high pressure loads are often associated with cardiac pathologies (Grossman 1980).

The thickness of the ventricular wall is closely linked to the mass of the heart and the size of the animal. Thus, the effect of volume and pressure loading on wall thickness is ultimately realized as variation in heart mass. Mammalian heart mass, therefore, has been the focus of several allometric analyses even if the underlying Laplacian principles have not always been appreciated. An allometric analysis of heart mass (M_h) against body mass (M_b) can be expressed in the form of a power equation, $M_h = a \cdot M_b^b$, where a is the scaling coefficient (elevation of the log-transformed equation) and b is the scaling exponent (slope of the log-transformed equation). If $b = 1$, then heart mass increases in direct proportion to body mass (isometry); if $b > 1$, then it increases faster than body mass (hyperallometry); and if $b < 1$, then it increases slower than body mass (hypoallometry; Calder 1996). Variation in mammalian heart mass due to factors independent of body mass, for example, athletic versus nonathletic species of similar size, is evident as variation on either side of the mean line describing the allometric function (Brody 1945; Poupa and Ošťádal 1969).

*This paper has been included in the Focused Issue on "Developmental Physiology" at the request of Theodore Garland Jr., Editor in Chief.

†Corresponding author; e-mail: edward.snelling@adelaide.edu.au.

It is generally accepted that mammalian heart mass scales in direct proportion to body mass across species (phylogenetic) based on a number of influential allometric analyses that arrive at an exponent of $b = 0.96\text{--}1.06$ (Brody 1945; Stahl 1965; Holt et al. 1968; Prothero 1979; Hoppeler et al. 1984; Bishop 1997; Seymour and Blaylock 2000; Lindstedt and Schaeffer 2002). Analyses restricted to marsupial mammals also trend toward isometry, $b = 0.94\text{--}1.05$ (Dawson and Needham 1981; Dawson et al. 2003). The isometric scaling of heart mass allows stroke volume to increase in parallel (Holt et al. 1968), while maximum stress across the wall is kept constant, at approximately 14 kPa in the case of the left ventricle (Seymour and Blaylock 2000). Likewise, it is also assumed that heart mass scales in direct proportion to body mass during mammalian growth and development (ontogenetic). However, this generalization lacks empirical support. It is true that mice and humans tend to show isometric heart growth across postnatal development (e.g., Thompson 1942; Hou and Burggren 1989), but in most mammal species studied to date, heart mass scales with hypoallometry across postnatal development, as shown for rats (Stewart and German 1999), guinea pigs (data in Webster and Liljegren 1949), dogs (Deavers et al. 1972), sheep, swine, horses, and cattle (data in Altman and Dittmer 1962). To add further complexity, biphasic scaling has been reported for humans and the giraffe *Giraffa camelopardalis*, where heart mass increases with a relatively steep exponent during prenatal development before “switching” to a shallow postnatal exponent around the time of parturition (Hirokawa 1972; Mitchell and Skinner 2009). The ability to detect two or more allometric phases of heart growth across development relies heavily on the quality of the data set: it might be easily missed if few developmental stages are incorporated into the allometric analysis, or if the analyzed body mass range is restricted. A narrow body mass range not only fails to incorporate the full scope of development, but it also runs the risk of mass-independent variation overwhelming the analysis, resulting in a derived exponent (b) that has broad confidence limits and offering little insight on the relation between body mass and heart size (Calder 1987). Certainly with respect to placental mammals, the narrow postnatal body mass range (ca. 20–50-fold) is unavoidable. However, the postnatal body mass range of marsupial mammals is considerably broader (ca. 1,000-fold) and provides the opportunity to undertake more accurate allometric analyses of cardiac growth.

The broad postnatal body mass range of marsupials arises from their unique mode of reproduction, which consists of a short gestation period, birth to undeveloped altricial young, followed by a protracted period of lactation and development, usually within a pouch (Renfree 2006). The anatomical changes that occur in the heart and major blood vessels when neonates shift to aerial respiration at birth are similar in marsupial and placental mammals, except that in marsupials it occurs much earlier in development. The ductus arteriosus shunt closes rapidly after birth, while the interatrial communication, which is in the form of a fenestrated septum, closes gradually over a few days, after which the pulmonary and systemic circulations

are completely separated, and the left and right ventricular outputs are balanced (Runciman et al. 1995). However, later in life, a number of structural and functional differences between the marsupial and placental heart become evident. The heart mass and stroke volume of marsupials are on average 130% of similarly sized placental mammals, while heart rate is around 50% of the placental value (Dawson and Needham 1981; Dawson et al. 2003). Thus, the cardiac output and metabolic rate of marsupials at rest is approximately 70% of the general value for placentals (Dawson and Hulbert 1969, 1970; Hayssen and Lacy 1985). Nonetheless, respirometry and morphometry both suggest that cardiac output and metabolic rate during heavy exercise are similar between the clades owing to the larger aerobic scope of marsupials (Kram and Dawson 1998; Dawson et al. 2003).

The tammar wallaby *Macropus eugenii* is the only marsupial on which an allometric analysis of heart growth has been undertaken (Hulbert et al. 1991). In this species, the mass of the whole heart increases in perfect isometry ($b = 1.00$) during postnatal development. Here, we undertake a similar analysis by examining the allometric scaling of whole-heart mass, whole-heart geometry, and the mass of the individual chambers of the heart across postnatal development in the marsupial western grey kangaroo *Macropus fuliginosus*. Both the tammar wallaby and western grey kangaroo engage in hopping (saltatory) locomotion; however, the western grey kangaroo grows an order of magnitude larger, occupies more open habitats suitable for sustained hopping, and is among the largest of the extant Australian macropod species. Moreover, the metabolic efficiency of hopping increases in larger species of macropod (Bennett 2000), and we can test the hypothesis that this might unload some of the work of the heart, which would be reflected in the allometric scaling of heart mass.

Material and Methods

Animal Collection

We accompanied a licensed marksman during a planned conservation cull of western grey kangaroos *Macropus fuliginosus* (Desmarest 1817), 80 km south of Adelaide, Australia. The marksman shot all animals according to Australian legal requirements following the *Code of Practice for the Humane Shooting of Kangaroos* (<http://www.environment.gov.au>). Approval to scavenge organ and tissue samples from animals euthanized for conservation, or other non-research-related purposes, was provided by the University of Adelaide Animal Ethics Committee (S-2011-223).

Heart Dissection

Body mass was determined by suspending the carcass from an appropriate spring balance (0.2-, 2.0-, 5.0-, 20-, or 100-kg capacity, Salter, Melbourne). The thoracic cavity was then opened, and the whole heart was removed, sealed in a plastic zipper-lock bag, stored under ice, and transported to the University of Adelaide. In the laboratory, congealed blood was

emptied from the chambers, the major blood vessels dissected free, and the heart rinsed clean with an isotonic saline solution (0.90% w/v NaCl). The height, width, and depth of each heart were measured to 0.01 mm with digital callipers and the average calculated. Then each heart was separated into left and right atrium and left and right ventricle. The interatrial septum was dissected as part of the right atrium, and the interventricular septum was dissected as part of the left ventricle, following what have become standard procedures (Fulton et al. 1952; Keen 1955; Joyce et al. 2004). Chambers <30 g were weighed to 0.1 mg (AE163, Mettler, Greifensee, Switzerland) and chambers >30 g were weighed to 1.0 mg (1265 MP, Sartorius, Göttingen, Germany), and then chamber masses were summed to provide total heart mass.

Statistical Analyses

All mean values are presented with 95% confidence intervals. Allometric relationships were derived by taking the $\log_{10}$ of the variable and the $\log_{10}$ of body mass and calculating ordinary least squares linear regressions. The slopes and intercepts of the regressions were compared using ANCOVA, with heart mass, chamber mass, or average heart dimension set as the dependent variable, and body mass as the covariate (Zar 1998), using statistical software (GraphPad Prism 6, GraphPad Software, USA). To determine whether biphasic allometry existed in the data set, a breakpoint analysis was performed by fitting a series of two-phase linear regressions to the $\log_{10}$ -transformed data of heart mass against body mass in which the intersection point was shifted along consecutive readings. The breakpoint was identified as the intersection that minimized the sum of the regressions' residual sums of squares (Yeager and Ultsch 1989; Mueller and Seymour 2011). An ANCOVA was then performed on the two regressions to test for statistical significance.

Results

Allometry of Heart Mass

Hearts obtained from 29 western grey kangaroos over an 800-fold body mass range (0.084–67.5 kg) weighed between 0.48% and 1.03% of body mass. Full allometric power equations describing heart and chamber mass as a function of body mass are presented in figures 1 and 2. Across all individuals, heart mass increases with body mass with an exponent of 1.03 ± 0.03 (fig. 1a). The 95% confidence interval includes 1.0 and is therefore consistent with isometry. However, two distinct regressions are evident in the data set (fig. 1b), and a breakpoint analysis confirms that the split between the regressions occurs at approximately 5–6 kg body mass, which is when joeys of this species typically leave the pouch (Dawson 2012). The heart mass of pouch joeys (hereafter termed “in-pouch”) scales with hyperallometry, with an exponent of 1.10 ± 0.05 , while the heart mass of free-roaming juveniles and adults (hereafter “postpouch”) scales with hypoallometry, with a significantly lower exponent of 0.77 ± 0.08 (ANCOVA, $F_{1,25} = 54.5$, $P < 0.0001$). The biphasic allometry of heart mass is also reflected

Figure 1. Allometric scaling of (a) whole-heart mass M_h throughout development in the marsupial western grey kangaroo *Macropus fuliginos* ($N = 29$). Also shown is the allometric scaling of (b) whole-heart mass in in-pouch joeys (open circles, $N = 11$) compared to free-roaming postpouch juveniles and adults (filled circles, $N = 18$). The allometric trajectory of heart growth decreases at ca. 5–6 kg body mass. Equations are in the form $y = a \cdot M_b^b$, where y is the variable of interest (in grams), a is the coefficient (elevation), b is the exponent (slope), and M_b is body mass (in kilograms). Dashed lines are 95% confidence limits for the regression mean. Coefficient of determination (r^2) and 95% confidence limits for the exponent are given. Dagger indicates in-pouch exponent significantly different from corresponding postpouch exponent (ANCOVA, $P < 0.05$).

in the scaling of the heart's linear dimensions. The mean of the heart's height, width, and depth ($\bar{L}_h$, mm) scales across pouch joeys according to $\bar{L}_{h(\text{in-pouch})} = 23.9 M_b^{0.37 \pm 0.03}$, whereas in free-roaming juveniles and adults it scales as $\bar{L}_{h(\text{postpouch})} = 30.2 M_b^{0.27 \pm 0.04}$ ($F_{1,25} = 19.5$, $P < 0.001$).

Allometry of Chamber Masses

The hyperallometry of in-pouch whole-heart mass is also apparent in the scaling exponent of in-pouch left ventricle wall + septum mass (1.13 ± 0.06 ; fig. 2a) and right ventricle wall mass (1.09 ± 0.05 ; fig. 2b), both of which are statistically indistinguishable from the scaling exponent for in-pouch whole-heart mass (ANCOVA, $F_{1,18} = 0.58$, $P = 0.46$; and $F_{1,18} = 0.019$, $P = 0.89$, respectively), but with significantly lower elevation ($F_{1,19} = 125$, $P < 0.0001$; and $F_{1,19} = 1,929$,

Figure 2. Allometric scaling of (a) left ventricle wall + septum mass M_{LV} , (b) right ventricle wall mass M_{RV} , (c) left atrium mass M_{LA} , and (d) right atrium mass M_{RA} in in-pouch joeys (open circles, $N = 11$) compared to free-roaming postpouch juvenile and adult (filled circles, $N = 18$) western grey kangaroos *Macropus fuliginosus*. Dagger indi-

$P < 0.0001$, respectively). In contrast, the scaling exponents of in-pouch left atrium mass (0.99 ± 0.07 ; fig. 2c) and right atrium mass (0.97 ± 0.07 ; fig. 2d) trend toward isometry and are both significantly lower than the exponent for in-pouch whole-heart mass ($F_{1,18} = 8.29$, $P < 0.01$; and $F_{1,18} = 11.0$, $P < 0.01$, respectively). The hypoallometry of postpouch whole-heart mass is also evident in the scaling exponent of postpouch left ventricle wall + septum mass (0.77 ± 0.09), right ventricle wall mass (0.76 ± 0.10), left atrium mass (0.82 ± 0.12), and right atrium mass (0.76 ± 0.08 ; fig. 2).

Discussion

In laying the foundations of allometry, Sir D'Arcy Wentworth Thompson concluded that the development of organisms is such a complex process that the uniform and constant growth of its parts would be "an unlikely and an unusual circumstance" (Thompson 1942). Life-history events that involve a change in the animal's behavior and physiology are bound to be reflected in its anatomy. In particular, shifts in thermoregulatory ability and intensity of locomotion are expected to affect metabolic rate and hence the oxygen demand that is met by the respiratory and circulatory systems. Our ontogenetic study of western grey kangaroos demonstrates such a shift over an 800-fold range of body mass, from an 84-g joey to a 64-kg adult (fig. 1b). Heart mass of pouch joeys scales with hyperallometry ($b = 1.10$), whereas in free-roaming juveniles and adults it scales with hypoallometry ($b = 0.77$). The shift in exponent occurs at approximately 5–6 kg body mass, which corresponds to pouch emergence in this species (Dawson 2012). Two other relevant changes occur across this transition: the achievement of endothermy and an increased requirement for independent terrestrial locomotion.

Biphasic Cardiac Allometry and the Shift from Ectothermy to Endothermy

Marsupials are born altricial and ectothermic, with mass-specific metabolic rates that are similar to those of adults at the same body temperature, despite the great difference in body size (Rose et al. 1998). Although marsupial neonates cannot generate their own heat, body temperature is nonetheless kept constant because of the stable temperature of the pouch (Shield 1966; Farber and Tenney 1971). From these ectothermic beginnings, the metabolic rate and heat-generating capacity of the joey gradually increase to the point that it becomes an effective endotherm toward the end of pouch life (Petajan and Morrison 1962; Shield 1966; Setchell 1974; Loudon et al. 1985; Hulbert 1988; Rose et al. 1998). Around this time, fur develops, mass-specific metabolic rates increase (Rose et al. 1998), and

cates in-pouch exponent significantly different from corresponding postpouch exponent (ANCOVA, $P < 0.05$). Asterisk indicates in-pouch exponent for left and right atrium significantly different from the in-pouch exponent for whole-heart mass ($P < 0.05$). See figure 1 for equation format.

the juvenile kangaroo is in a physiological position to continue development out of the pouch. It is relevant that the ontogenetic scaling of resting metabolic rate of marsupials follows a biphasic allometry, with the breakpoint occurring when the joey leaves the pouch. For example, the resting metabolic rate of bettongs *Bettongia gaimardi* (small bipedal macropod marsupials) scales with an exponent of 1.15 while in the pouch but drops to 0.63 after pouch exit (Rose et al. 1998). Similarly, oxygen consumption rates of resting tammar wallabies *Macropus eugenii*, from birth at 360 mg to adulthood at 8 kg body mass, show a reduction in allometric exponent, from 0.82 in ectothermic joeys to 0.75 in endothermic joeys at foot (Frappell 2008). There are similar changes in the scaling of ventilation rate in the tammar wallaby, and assuming a constant ventilation : perfusion ratio, cardiac output would also scale biphasically (Frappell 2008). Equivalent studies on western grey kangaroos have not been published; however, a biphasic scaling pattern in oxygen demand in this species would explain the biphasic scaling we observe in heart mass, since the mass of the heart is closely related to the circulatory work it is required to perform. The shallow heart mass exponent following pouch vacation ($b = 0.77$) might be partly related to the decreasing ratio of surface area to body mass with growth, which could unburden some of the cardiac load associated with the maintenance of body temperature when ambient temperatures drop well below the thermal neutral zone.

Biphasic Cardiac Allometry and the Transition to Independent Locomotion

In addition to the demands of endothermy, there is also a steady increase in locomotory capabilities as the joey makes the transition from pouch to independent life. Young pouch joeys are permanently attached to the teat and show limited movement as their hind legs are practically nonexistent (Sharman and Pilton 1964), but as development progresses, the legs become longer and muscular (Poole et al. 1985) until the joey begins to venture tentatively from the pouch (Dawson 2012). The gradual increase in overall motility is probably important, because once joeys vacate the pouch, they have to hop as fast as their mothers. Thus, the steep scaling exponent of heart mass during in-pouch development ($b = 1.10$) might be partly associated with the increasing locomotory motility and the attainment of a high level of aerobic capacity necessary after pouch vacation.

The marked decrease in exponent after pouch exit ($b = 0.77$) is possibly associated with the fact that hopping locomotion is energetically more efficient as body size increases. In the hopping mode, speed becomes independent of metabolic rate because of the relatively high efficiency of elastic energy storage in the muscles, ligaments, and tendons of the hind limbs and tail between each hop (Dawson and Taylor 1973; Baudinette et al. 1992). This storage increases with body mass, which accounts for the fact that, while metabolic rate is independent of increases in speed in large macropods, there is no uncoupling of metabolic rate from speed in small macropods (Baudinette

1991, 1994; Bennett and Taylor 1995; Bennett 2000). It is reasonable to expect that heart mass ultimately depends more on maximum aerobic metabolic rate than resting metabolic rate. Therefore, an increasing efficiency of hopping with larger body size would be expected to result in a shallow slope of the allometric line for heart mass. If an ontogenetic trade-off exists between relative heart size and the capacity to store energy during hopping locomotion, then this should be evident, from a functional perspective, as a shallow exponent for hopping metabolic rate across the postpouch phase of development. However, owing to difficulties associated with training large kangaroos (6–70 kg body mass) to hop at high speed on inclined treadmills (Kram and Dawson 1998), these data have not yet been assembled.

Cardiac Allometry in Other Mammal Species

Previous allometric studies of heart growth in mammals typically report a single constant exponent across development, between $b = 0.7$ and 1.0 (table 1). The wide range of reported exponents suggests that the trajectory of heart growth following birth varies significantly between species. This might be influenced by the endothermic capacity and metabolic requirements of neonates at birth, the level of cardiomyocyte maturity at birth, the athletic ability of the adult animal, and potentially the scaling of other large organs in the body. Nonetheless, most of the previous studies on heart growth allometry are restricted to changes over a limited range of body size that occurs postnatally. The few studies that have encompassed both pre- and postnatal development, however, generally report biphasic heart growth allometry, supporting the idea that the development of endothermy and the associated increase in cardiac demands are related. The heart mass of humans (Hirokawa 1972) and giraffes (Mitchell and Skinner 2009) both increase with relatively steep exponents during prenatal growth before shifting to shallow exponents after birth. The notable exception is a study of whole-heart mass in the tammar wallaby, which scales uniformly with body mass to the 1.0 power over a 1,550-fold body mass range (Hulbert et al. 1991). Why a break in exponent is not apparent in this hopping macropod is unclear, but it might be related to the one-tenth smaller maximum size of this animal (ca. 7 kg body mass) compared to the western grey kangaroo (ca. 70 kg). Other species-specific differences might also contribute to the different cardiac scaling in these two macropods. For instance, the more open habitats occupied by kangaroos might allow them to better exploit the energy recovery system of the hind legs and thus off-load a greater proportion of work from the heart during high-speed sustained hopping.

The Right : Left Ventricular Mass Ratio

Our study appears to be the first to include an allometric analysis of growth of the individual chambers of the heart (fig. 2). An examination of the scaling of each chamber provides an indication of how pressure loading, volume loading,

Table 1: Allometric exponents for heart mass across development in mammals

Mammal and life stage	Exponent (b) ^a	Body mass range	Source
Mouse:			
Postnatal	1.00 ($\pm .09$)	24-fold; 1.4–33 g	1
Postnatal	.97 ($\pm .06$)	43-fold; 1.0–43 g	2
Rat:			
Postnatal	.82	40-fold; 10–400 g	3
Postnatal	.80 ($\pm .02$)	86-fold; 5.3–454 g	4
Postnatal	.78 ($\pm .04$)	112-fold; 5.8–647 g	5
Postnatal	.74	50-fold; 15–750 g	6
Guinea pig:			
Postnatal	.74 ($\pm .05$)	17-fold; 77–1,319 g	7
Wallaby: ^{b,c}			
Postnatal	1.00	1,550-fold; .004–6.2 kg	8
Dog:			
Postnatal	.91	53-fold; .20–10.5 kg	9
Postnatal	.91 ($\pm .09$)	15-fold; 2.4–37 kg	10
Sheep:			
Postnatal	.66 ($\pm .11$)	6.8-fold; 5.7–39 kg	1
Kangaroo: ^b			
In-pouch	1.10 ($\pm .05$)	49-fold; .084–4.1 kg	This study
Postpouch	.77 ($\pm .08$)	9.0-fold; 7.5–67.5 kg	This study
Human: ^d			
Prenatal	1.19	4-fold; 1–4 kg	11
Postnatal	.89	16.7-fold; 3–50 kg	11
Human (1–30 yr old):			
Postnatal	1.02 ($\pm .03$)	6.9-fold; 8.8–61 kg	12
Human (1–25 yr old):			
Postnatal	1.01 ($\pm .04$)	7.4-fold; 9–66 kg	13
Human (.01–85 yr old): ^e			
Postnatal	1.00	25-fold; 4–98 kg	14
Swine:			
Postnatal	.76 ($\pm .12$)	75-fold; 1.3–100 kg	1
Horse:			
Postnatal	.89 ($\pm .06$)	34-fold; 13–446 kg	1
Cattle:			
Postnatal	.79 ($\pm .19$)	11-fold; 52–597 kg	1
Postnatal	.90 ($\pm .07$)	9.9-fold; 90–888 kg	1
Giraffe:			
Prenatal	1.03	4.3-fold; 18–77 kg	15
Postnatal	.90	80-fold; 147–1,441 kg	15

Sources. 1, Calculated from data in Altman and Dittmer 1962; 2, Hou and Burggren 1989; 3, von Bertalanffy and Pirozynski 1952; 4, calculated from data in Hatai 1913; 5, calculated from data in David et al. 1981; 6, Stewart and German 1999; 7, calculated from data in Webster and Liljegren 1949; 8, Hulbert et al. 1991; 9, Deavers et al. 1972; 10, calculated from data in Stewart 1921; 11, Hirokawa 1972; 12, calculated by Rakusan (1980) from data in Mühlmann 1927; 13, calculated from data in Thompson 1942; 14, de Simone et al. 1998; 15, Mitchell and Skinner 2009.

^aWhere possible, the $\pm 95\%$ confidence interval is presented.

^bMarsupial.

^cRegression across in-pouch and postpouch wallaby development.

^dSummed left and right ventricle mass only.

^eLeft ventricle mass only.

and the different functional tasks of each chamber vary across development. It is particularly interesting that the right and left ventricle masses scale in parallel across in-pouch and postpouch life (fig. 2*a*, 2*b*), exhibiting a relatively fixed ratio: a 1-kg pouch joey has a right : left ventricular mass ratio of 0.30, and in a 30-kg free-roaming kangaroo, the ratio is 0.35. These ventricular mass ratios are similar to data for other mammals measured in the same way, including 0.33–0.37 in adult humans (Keen 1955; Sandstede et al. 2000) and 0.36 in greyhound dogs (Bienvenu and Drolet 1991). The ventricular mass ratio is higher in mammalian fetuses because pulmonary and systemic blood pressures are similar, but following birth, the ratio decreases quickly as pulmonary resistance decreases. In humans, for example, the ratio decreases from 0.8 at birth to 0.4 by 3 mo of age (Keen 1955; Joyce et al. 2004). This postnatal change in ventricular mass ratio is not evident in our data because the youngest joey had been breathing air for approximately 3 mo, as estimated from its body mass of 85 g (Poole et al. 1985), and smaller animals ought to develop faster than human neonates.

The external work of the heart is related to the product of blood flow rate (cardiac output) and blood pressure. Given that cardiac output is the same in the two ventricles, we can examine the relationship between the pulmonary : systemic blood pressure ratio and the right : left ventricular mass ratio. If the geometry of the two ventricles were identical, we would expect to see muscle masses in proportion to blood pressures in the two chambers. The adult kangaroo, however, has a mean pulmonary pressure of 13.3 mmHg and a mean systemic pressure of 92.0 mmHg (Maxwell et al. 1964), which equates to a pulmonary : systemic blood pressure ratio of 0.14. This blood pressure ratio is comparable to the average ratio (0.16 ± 0.03) calculated from 11 mammal species sourced from the literature (table 2). Clearly, these blood pressure ratios are significantly less than the right : left ventricular mass ratios observed in this and other studies of mammals (0.30–0.35). The explanation for the disparity is another example of the principle of Laplace. Because the right ventricle appears as a partial shell around the left ventricle, its radius of curvature is necessarily larger (Mirsky 1974; Huisman et al. 1980). This geometry puts the right ventricle at a disadvantage to developing pressure, and consequently the wall must be thicker.

Application of the principle of Laplace assumes that the cross-sectional tension (= stress) produced by cardiac muscle is a constant. While this may be generally true interspecifically among mammals and birds (Seymour and Blaylock 2000), maturation of cardiac ultrastructure, particularly during early development, requires that some caution is taken before applying the principle ontogenetically. It is well established that the volume density of the myofibril contractile machinery increases during fetal development in placental mammals (Canale et al. 1986), and the tension developed by isolated cardiac muscle performing isometric contraction also increases over this period (Friedman 1972). It is reasonable to suspect that similar changes to cardiac ultrastructure would also occur during in-pouch growth in marsupial mammals.

Table 2: Mean pulmonary and systemic arterial blood pressures (mmHg) and the pulmonary-systemic pressure ratio in mammals

	Pulmonary	Systemic	Ratio	Reference
Mouse	27.0	111	.24	1
Rat	18.8	111	.17	2
Cat ^b	19.5	118	.17	3,4
Pademelon ^a	7.3	126	.06	5
Dog ^b	14.7	113	.13	6–12
Sheep ^b	16.3	94.3	.17	13–18
Kangaroo ^a	13.3	92.0	.14	5
Human	15.0	93.0	.16	19
Swine	15.0	104	.14	20
Calf ^b	27.6	110	.25	21–24
Horse ^b	20.4	120	.17	25, 26

Sources. 1, Thibault et al. 2010; 2, Smith and Bennett 1934; 3, von Euler and Liljestrand 1946; 4, Arvidsson et al. 1983; 5, Maxwell et al. 1964; 6, Mercier et al. 2010; 7, Rippe et al. 1982; 8, Sundet et al. 1984; 9, Shelub et al. 1984; 10, Haskins et al. 1986*a*; 11, Haskins et al. 1986*b*; 12, Ilkiw et al. 1992; 13, Hanaoka et al. 1999; 14, Kubo et al. 1982; 15, Nesarajah et al. 1983; 16, Matalon et al. 1982; 17, Matalon et al. 1983; 18, Gratama et al. 1990; 19, Rhoades and Bell 2009; 20, Tranquilli et al. 1982; 21, Gross et al. 1981; 22, Gross et al. 1979; 23, Manohar et al. 1982; 24, Lin et al. 1991; 25, Dixon 1978; 26, Manohar et al. 1981.

Note. There was no systematic effect of body mass.

^aMarsupial.

^bAveraged from multiple studies.

Conclusion

In summary, the heart of the western grey kangaroo follows a biphasic allometric growth pattern. During the in-pouch phase of development, heart mass increases with a relatively steep exponent ($b = 1.10$), which is consistent with the increase in relative cardiac demands as joeys develop endothermy and increase their capacity for hopping locomotion. Once independence from the pouch is achieved (ca. 5–6 kg body mass), heart growth in free-roaming kangaroos switches to a shallow exponent ($b = 0.77$), possibly because the energy required for hopping is independent of speed, and the efficiency of energy storage during hopping increases as the kangaroo grows. These findings thus offer support to our hypothesis that the increasing metabolic efficiency of hopping locomotion in larger macropods might reduce some of the work of the heart.

Acknowledgments

We thank Dr. David McLelland from Zoos South Australia, who made us aware of the hemodynamic study by Maxwell and colleagues. We acknowledge the exceptional and humane marksmanship exhibited during the conservation cull, and we thank the marksman for allowing access to carcasses. We are indebted to volunteers who assisted in the field. We thank John Snelling from the University of Adelaide for the donation of a utility vehicle to collect carcasses. We also thank Associate Professor Craig White from the University of Queensland for

providing the macro to undertake the breakpoint analysis. Two reviewers provided valuable feedback on the manuscript. This research was supported by the Australian Research Council (DP120102081).

Literature Cited

- Altman P.L. and D.S. Dittmer. 1962. Growth including reproduction and morphological development. Biological Handbook Series. Federation of American Societies for Experimental Biology, Washington, DC.
- Arvidsson S., A. Falk, E. Haglund, and U. Haglund. 1983. The role of 5-hydroxytryptamine in the feline response to intravenous infusion of live *E. coli*. *Br J Pharmacol* 79:711–718.
- Baudinette R.V. 1991. The energetics and cardiorespiratory correlates of mammalian terrestrial locomotion. *J Exp Biol* 160:209–231.
- . 1994. Locomotion in macropodoid marsupials: gaits, energetics and heat balance. *Aust J Zool* 42:103–123.
- Baudinette R.V., G.K. Snyder, and P.B. Frappell. 1992. Energetic cost of locomotion in the tammar wallaby. *Am J Physiol* 262:R771–R778.
- Bennett M.B. 2000. Unifying principles in terrestrial locomotion: do hopping Australian marsupials fit in? *Physiol Biochem Zool* 73:726–735.
- Bennett M.B. and G.C. Taylor. 1995. Scaling of elastic strain energy in kangaroos and the benefits of being big. *Nature* 378:56–59.
- Bienvenu J.-G. and R. Drolet. 1991. A quantitative study of cardiac ventricular mass in dogs. *Can J Vet Res* 55:305–309.
- Bishop C.M. 1997. Heart mass and the maximum cardiac output of birds and mammals: implications for estimating the maximum aerobic power input of flying animals. *Philos Trans R Soc B* 352:447–456.
- Brody S. 1945. Bioenergetics and growth: with special reference to the efficiency complex in domestic animals. Reinhold, New York.
- Calder W.A., III. 1987. Scaling energetics of homeothermic vertebrates: an operational allometry. *Annu Rev Physiol* 49: 107–120.
- . 1996. Size, function, and life history. Dover, New York.
- Canale E.D., G.R. Campbell, J.J. Smolich, and J.H. Campbell. 1986. Cardiac muscle. Springer, Berlin.
- David H., A. Bóznér, R. Meyer, and G. Wassilew. 1981. Pre- and postnatal development and ageing of the heart: ultrastructural results and quantitative data. *Exp Pathol Suppl* 7:1–176.
- Dawson T.J. 2012. Kangaroos. 2nd ed. CSIRO, Collingwood, Melbourne.
- Dawson T.J. and A.J. Hulbert. 1969. Standard energy metabolism of marsupials. *Nature* 221:383.
- . 1970. Standard metabolism, body temperature, and surface areas of Australian marsupials. *Am J Physiol* 218: 1233–1238.
- Dawson T.J. and A.D. Needham. 1981. Cardiovascular characteristics of two resting marsupials: an insight into the cardio-respiratory allometry of marsupials. *J Comp Physiol* 145:95–100.
- Dawson T.J. and C.R. Taylor. 1973. Energetic cost of locomotion in kangaroos. *Nature* 246:313–314.
- Dawson T.J., K.N. Webster, B. Mifsud, E. Raad, E. Lee, and A.D. Needham. 2003. Functional capacities of marsupial hearts: size and mitochondrial parameters indicate higher aerobic capabilities than generally seen in placental mammals. *J Comp Physiol B* 173:583–590.
- Deavers S., R.A. Huggins, and E.L. Smith. 1972. Absolute and relative organ weights of the growing beagle. *Growth* 36: 195–208.
- de Simone G., R.B. Devereux, T.R. Kimball, G.F. Mureddu, M.J. Roman, F. Contaldo, and S.R. Daniels. 1998. Interaction between body size and cardiac workload: influence on left ventricular mass during body growth and adulthood. *Hypertension* 31:1077–1082.
- Dixon P.M. 1978. Pulmonary artery pressures in normal horses and in horses affected with chronic obstructive pulmonary disease. *Equine Vet J* 10:195–198.
- Farber J.P. and S.M. Tenney. 1971. The pouch gas of the Virginia opossum (*Didelphis virginiana*). *Respir Physiol* 11:335–345.
- Ford L.E. 1976. Heart size. *Circ Res* 39:297–303.
- Frappell P.B. 2008. Ontogeny and allometry of metabolic rate and ventilation in the marsupial: matching supply and demand from ectothermy to endothermy. *Comp Biochem Physiol A* 150:181–188.
- Friedman W.F. 1972. The intrinsic physiologic properties of the developing heart. *Prog Cardiovasc Dis* 15:87–111.
- Fulton R.M., E.C. Hutchinson, and A.M. Jones. 1952. Ventricular weight in cardiac hypertrophy. *Br Heart J* 14:413–420.
- Grande F. and H.L. Taylor. 1965. Adaptive changes in the heart, vessels, and patterns of control under chronically high loads. Pp. 2615–2677 in W.F. Hamilton, ed. *Handbook of Physiology*. Sec. 2. Circulation. Vol. 3. American Physiological Society, Washington, DC.
- Gratama J.W.C., J.J. Meuzelaar, M. Dalinghaus, J.H. Koers, A.J. Werre, W.G. Zijlstra, and J.R.G. Kuipers. 1990. Maximal exercise capacity and oxygen consumption of lambs with an aortopulmonary left-to-right shunt. *J Appl Physiol* 69:1479–1485.
- Gross D.R., K.T. Dodd, J.D. Williams, and H.R. Adams. 1981. Adverse cardiovascular effects of oxytetracycline preparations and vehicles in intact, awake calves. *Am J Vet Res* 42: 1371–1377.
- Gross D.R., J.V. Kitzman, and H.R. Adams. 1979. Cardiovascular effects of intravenous administration of propylene glycol and of oxytetracycline in propylene glycol in calves. *Am J Vet Res* 40:783–791.
- Grossman W. 1980. Cardiac hypertrophy: useful adaptation or pathological process? *Am J Med* 69:576–584.
- Hanaoka M., K. Kubo, T. Hayano, T. Koizumi, and T. Kobayashi. 1999. Interferon- α elevates pulmonary blood pressure in sheep: the role of thromboxane cascade. *Eur J Pharmacol* 370:145–151.

- Haskins S.C., T.B. Farver, and J.D. Patz. 1986a. Cardiovascular changes in dogs given diazepam and diazepam-ketamine. *Am J Vet Res* 47:795–798.
- Haskins S.C., J.D. Patz, and T.B. Farver. 1986b. Xylazine and xylazine-ketamine in dogs. *Am J Vet Res* 47:636–641.
- Hatai S. 1913. On the weights of the abdominal and the thoracic viscera, the sex glands, ductless glands and the eyeballs of the albino rat (*Mus norvegicus albinus*) according to body weight. *Am J Anat* 15:87–119.
- Hayssen V. and R.C. Lacy. 1985. Basal metabolic rates in mammals: taxonomic differences in the allometry of BMR and body mass. *Comp Biochem Physiol A* 81:741–754.
- Hirokawa K. 1972. A quantitative study on pre- and postnatal growth of human heart. *Acta Pathol Jpn* 22:613–624.
- Holt J.P., E.A. Rhode, and H. Kines. 1968. Ventricular volumes and body weight in mammals. *Am J Physiol* 215:704–715.
- Hoppeler H., S.L. Lindstedt, H. Claassen, C.R. Taylor, O. Mathieu, and E.R. Weibel. 1984. Scaling mitochondrial volume in heart to body mass. *Respir Physiol* 55:131–137.
- Hou P.C.L. and W.W. Burggren. 1989. Interaction of allometry and development in the mouse *Mus musculus*: heart rate and hematology. *Respir Physiol* 78:265–280.
- Huisman R.M., P. Sipkema, N. Westerhof, and G. Elzinga. 1980. Comparison of models used to calculate left ventricular wall force. *Med Biol Eng Comput* 18:133–144.
- Hulbert A.J. 1988. Metabolism and the development of endothermy. Pp. 148–161 in C.H. Tyndale-Biscoe and P.A. Janssens, eds. *The developing marsupial: models for biomedical research*. Springer, Berlin.
- Hulbert A.J., W. Mantaj, and P.A. Janssens. 1991. Development of mammalian endothermic metabolism: quantitative changes in tissue mitochondria. *Am J Physiol* 261:R561–R568.
- Ilkiw J.E., P.J. Pascoe, S.C. Haskins, and J.D. Patz. 1992. Cardiovascular and respiratory effects of propofol administration in hypovolemic dogs. *Am J Vet Res* 53:2323–2327.
- Joyce J.J., P.I. Dickson, N. Qi, J.E. Noble, J.U. Raj, and B.G. Baylen. 2004. Normal right and left ventricular mass development during early infancy. *Am J Cardiol* 93:797–801.
- Keen E.N. 1955. The postnatal development of the human cardiac ventricles. *J Anat* 89:485–502.
- Kram R. and T.J. Dawson. 1998. Energetics and biomechanics of locomotion by red kangaroos (*Macropus rufus*). *Comp Biochem Physiol B* 120:41–49.
- Kubo K., T. Kobayashi, K. Yoshimura, M. Fukushima, K. Handa, S. Kusama, A. Sakai, and G. Ueda. 1982. Hemodynamic responses to prostacyclin (PGI₂) in the conscious sheep. *Jpn Circ J* 46:1292–1304.
- Lin H.C., J.C. Thurmon, W.J. Tranquilli, G.J. Benson, and W.A. Olson. 1991. Hemodynamic response of calves to tiletamine-zolazepam-xylazine anesthesia. *Am J Vet Res* 52:1606–1610.
- Lindstedt S.L. and P.J. Schaeffer. 2002. Use of allometry in predicting anatomical and physiological parameters of mammals. *Lab Anim* 36:1–19.
- Loudon A., N. Rothwell, and M. Stock. 1985. Brown fat, thermogenesis and physiological birth in a marsupial. *Comp Biochem Physiol A* 81:815–819.
- Manohar M., G.E. Bisgard, V. Bullard, and J.H.G. Rankin. 1981. Blood flow in the hypertrophied right ventricular myocardium of unanesthetized ponies. *Am J Physiol* 240: H881–H888.
- Manohar M., C.M. Parks, M.A. Busch, W.J. Tranquilli, G.E. Bisgard, T.A. McPherron, and M.C. Theodorakis. 1982. Regional myocardial blood flow and coronary vascular reserve in unanesthetized young calves exposed to a simulated altitude of 3500 m for 8–10 weeks. *Circ Res* 50:714–726.
- Matalon S., M.S. Nesarajah, and L.E. Farhi. 1982. Pulmonary and circulatory changes in conscious sheep exposed to 100% O₂ at 1 ATA. *J Appl Physiol* 53:110–116.
- Matalon S., M.S. Nesarajah, J.A. Krasney, and L.E. Farhi. 1983. Effects of acute hypercapnia on the central and peripheral circulation of conscious sheep. *J Appl Physiol* 54:803–808.
- Maxwell G.M., R.B. Elliott, and G.M. Kneebone. 1964. Hemodynamics of kangaroos and wallabies. *Am J Physiol* 206: 967–970.
- Mercier E., M. Mathieu, C.F. Sandersen, F.H. Delvaux, C.M. Clercx, and K. Mc Entee. 2010. Evaluation of the influence of age on pulmonary arterial pressure by use of right ventricular catheterization, pulsed-wave Doppler echocardiography, and pulsed-wave tissue Doppler imaging in healthy beagles. *Am J Vet Res* 71:891–897.
- Mirsky I. 1974. Review of various theories for the evaluation of left ventricular wall stress. Pp. 381–409 in I. Mirsky, D.N. Ghista, and H. Sandler, eds. *Cardiac mechanics: physiological, clinical, and mathematical considerations*. Wiley, New York.
- Mitchell G. and J.D. Skinner. 2009. An allometric analysis of the giraffe cardiovascular system. *Comp Biochem Physiol A* 154:523–529.
- Mueller C.A. and R.S. Seymour. 2011. The regulation index: a new method for assessing the relationship between oxygen consumption and environmental oxygen. *Physiol Biochem Zool* 84:522–532.
- Mühlmann M. 1927. Wachstum, Altern und Tod. *Ergeb Anat Entwicklungsgesch* 27:1–245.
- Nesarajah M.S., S. Matalon, J.A. Krasney, and L.E. Farhi. 1983. Cardiac output and regional oxygen transport in the acutely hypoxic conscious sheep. *Respir Physiol* 53:161–172.
- Petajan J.H. and P. Morrison. 1962. Physical and physiological factors modifying the development of temperature regulation in the opossum. *J Exp Zool* 149:45–57.
- Poole W.E., S.M. Carpenter, and J.T. Wood. 1985. Growth of grey kangaroos and the reliability of age determination from body measurements. II. The western grey kangaroos, *Macropus fuliginosus fuliginosus*, *M. f. melanops* and *M. f. ocydromus*: a correction. *Aust Wildl Res* 12:561–562.
- Poupa O. and B. Oštádal. 1969. Experimental cardiomegalies and “cardiomegalies” in free-living animals. *Ann N Y Acad Sci* 156:445–468.

- Prothero J. 1979. Heart weight as a function of body weight in mammals. *Growth* 43:139–150.
- Rakusan K. 1980. Postnatal development of the heart. Pp. 301–348 in G.H. Bourne, ed. *Hearts and heart-like organs*. Vol. 1. Comparative anatomy and development. Academic Press, New York.
- Renfree M.B. 2006. Society for Reproductive Biology Founders' Lecture 2006: life in the pouch: womb with a view. *Reprod Fertil Dev* 18:721–734.
- Rhoades R.A. and D.R. Bell. 2009. *Medical physiology: principles for clinical medicine*. 3rd ed. Lippincott Williams & Wilkins, Baltimore.
- Rippe J.M., L.A. Pape, J.S. Alpert, I.S. Ockene, J.A. Paraskos, P. Kotilainen, J. Anas, and W. Webster. 1982. Studies of systolic mechanics and diastolic behavior of the left ventricle in the trained racing greyhound. *Basic Res Cardiol* 77: 619–644.
- Rose R.W., N. Kuswanti, and E.Q. Colquhoun. 1998. Development of endothermy in a Tasmanian marsupial, *Bettongia gaimardi* and its response to cold and noradrenaline. *J Comp Physiol B* 168:359–363.
- Runciman S.I.C., B.J. Gannon, and R.V. Baudinette. 1995. Central cardiovascular shunts in the perinatal marsupial. *Anat Rec* 243:71–83.
- Sandstede J., C. Lipke, M. Beer, S. Hofmann, T. Pabst, W. Kenn, S. Neubauer, and D. Hahn. 2000. Age- and gender-specific differences in left and right ventricular cardiac function and mass determined by cine magnetic resonance imaging. *Eur Radiol* 10:438–442.
- Setchell P.J. 1974. The development of thermoregulation and thyroid function in the marsupial *Macropus eugenii* (Desmarest). *Comp Biochem Physiol A* 47:1115–1121.
- Seymour R.S. and A.J. Blaylock. 2000. The principle of Laplace and scaling of ventricular wall stress and blood pressure in mammals and birds. *Physiol Biochem Zool* 73:389–405.
- Sharman G.B. and P.E. Pilton. 1964. The life history and reproduction of the red kangaroo (*Megaleia rufa*). *Proc Zool Soc Lond* 142:29–48.
- Shelub I., A. van Grondelle, R. McCullough, S. Hofmeister, and J.T. Reeves. 1984. A model of embolic chronic pulmonary hypertension in the dog. *J Appl Physiol* 56:810–815.
- Shield J. 1966. Oxygen consumption during pouch development of the macropod marsupial *Setonix brachyurus*. *J Physiol* 187:257–270.
- Smith F.J.C. and G.A. Bennett. 1934. The pulmonary arterial pressure in normal albino rats and the effect thereon of epinephrine. *J Exp Med* 59:173–180.
- Stahl W.R. 1965. Organ weights in primates and other mammals. *Science* 150:1039–1042.
- Stewart G.N. 1921. Possible relations of the weight of the lungs and other organs to body-weight and surface area (in dogs). *Am J Physiol* 58:45–52.
- Stewart S.A. and R.Z. German. 1999. Sexual dimorphism and ontogenetic allometry of soft tissues in *Rattus norvegicus*. *J Morphol* 242:57–66.
- Sundet W.D., B.C. Wang, M.O.K. Hakumäki, and K.L. Goetz. 1984. Cardiovascular and renin responses to vanadate in the conscious dog: attenuation after calcium channel blockade. *Proc Soc Exp Biol Med* 175:185–190.
- Thibault H.B., B. Kurtz, M.J. Raheer, R.S. Shaik, A. Waxman, G. Derumeaux, E.F. Halpern, K.D. Bloch, and M. Scherrer-Crosbie. 2010. Noninvasive assessment of murine pulmonary arterial pressure: validation and application to models of pulmonary hypertension. *Circ Cardiovasc Imaging* 3:157–163.
- Thompson D.W. 1942. *On growth and form: a new edition*. Cambridge University Press, Cambridge.
- Tranquilli W.J., C.M. Parks, J.C. Thurmon, G.J. Benson, G.D. Koritz, M. Manohar, and M.C. Theodorakis. 1982. Organ blood flow and distribution of cardiac output in nonanesthetized swine. *Am J Vet Res* 43:895–897.
- von Bertalanffy L. and W.J. Pirozynski. 1952. Ontogenetic and evolutionary allometry. *Evolution* 6:387–392.
- von Euler U.S. and G. Liljestrand. 1946. Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol Scand* 12:301–320.
- Webster S.H. and E.J. Liljegren. 1949. Organ : bodyweight ratios for certain organs of laboratory animals. II. Guinea pig. *Am J Anat* 85:199–230.
- Yeager D.P. and G.R. Ultsch. 1989. Physiological regulation and conformation: a BASIC program for the determination of critical points. *Physiol Zool* 62:888–907.
- Zar J.H. 1998. *Biostatistical analysis*. Prentice Hall, Englewood Cliffs, NJ.