

RESEARCH ARTICLE

A meta-analysis of *in vivo* vertebrate cardiac performance: implications for cardiovascular support in the evolution of endothermy

Stanley S. Hillman^{1,*} and Michael S. Hedrick²

ABSTRACT

Endothermy in birds and mammals is associated with high body temperatures, and high rates of metabolism that are aerobically supported by elevated rates of cardiovascular O₂ transport. The purpose of this meta-analysis was to examine cardiovascular data from ectothermic and endothermic vertebrates, at rest and during exercise, with the goal of identifying key variables that may have contributed to the role of the cardiovascular system in supporting high rates of O₂ transport associated with endothermy. Vascular conductance, cardiac power and stroke work were summarized and calculated from a variety of studies at rest and during exercise for five classes of vertebrates where data were available. Conductance and cardiac power were linearly related to cardiac output from rest to exercise and also interspecifically. Exercise cardiac power and stroke work were greater in the endothermic species, owing to increased flow resulting from increased heart rate and increased pressure. Increased relative ventricle mass (RVM) was related to increased stroke volume in both groups. However, the increased RVM of endotherms was related to the increased pressure, as stroke work per gram of ventricle during exercise was equivalent between the groups. Cardiac power was linearly related to aerobic metabolic power, with 158 mW aerobic power output achieved per mW of cardiac power input. This analysis indicates that the greatly increased heart rate and cardiac stroke work leading to increased blood flow rate and blood pressure was necessary to support the metabolic requirements of endothermy.

KEY WORDS: Cardiac power, Stroke work, Vascular conductance, Vertebrate exercise, Exercise metabolism

INTRODUCTION

Endothermic vertebrates have maximal rates of aerobic metabolism about 10 times greater than those of ectotherms. Although a variety of hypotheses have been advanced to explain the evolution of endothermy from ectothermic ancestors (Cowles, 1958; McNab, 1978), the most widely accepted hypothesis is that endothermy occurred with selection of enhanced aerobic scope (Bennett and Ruben, 1979). A wealth of experimental evidence suggests that the systemic oxygen transport cardiovascular step of the oxygen cascade is the primary limitation to maximal rates of metabolism in fish (Kiceniuk and Jones, 1977; Gallaughan et al., 2001), amphibians (Withers and Hillman, 1988), reptiles (Frappell et al.,

2002), birds (Bishop, 1999), mammals (Andersen and Saltin, 1985; Powers et al., 1989; di Prampero, 2003) and vertebrates as a group (Hillman et al., 2013). This suggests that selection for enhanced aerobic capacity would act primarily on the cardiovascular system; however, it is not clear to what extent the cardiovascular system has contributed to, and supported, the evolution of endothermy from ectothermic ancestors.

It has also been suggested that the increased metabolism of endotherms is due to mitochondrial oxidative capacity of muscle providing the upper limit to maximal rates of aerobic metabolism (Weibel et al., 2004; Weibel and Hoppeler, 2005; Dawson et al., 2013). More recently, Clarke and Pörtner (2010) proposed a modification of the aerobic capacity hypothesis suggesting that an increase in body temperature was the means by which aerobic scope was achieved. This hypothesis posits that the key development in the evolution of endothermic metabolism was the proliferation and modification of mitochondria to achieve high body temperatures in conjunction with the ability to retain heat with insulation. However, this scenario implies there is no rate-limiting step in the delivery of O₂ to the mitochondria because any modification of mitochondrial capacity cannot provide an increase in aerobic power generation without a concomitant increase in O₂ supply to the mitochondria. In other words, mitochondrial capacity is independent of the diffusive and convective processes involved in moving oxygen from the environment to the mitochondria. Because blood flow rate is determined by the power output of the vertebrate heart, any increase in delivery of O₂ to mitochondria requires an enhancement of cardiac power. Consequently, a full understanding of ectotherm–endotherm aerobic power outputs requires an understanding of cardiac power inputs and how they compare between endotherms and ectotherms.

Maximal rates of aerobic metabolism ($\dot{V}_{O_{2,max}}$), the rate-limiting state, occur during intense exercise. Systemic oxygen transport (SOT=arterial blood O₂ content×systemic blood flow rate) appears to be the rate-limiting step to $\dot{V}_{O_{2,max}}$ in vertebrates (Hillman et al., 2013). Variation in blood flow rate is related to both the intrinsic capacity of the heart to do work and the characteristics of the vasculature to enhance (or impede) flow. In this approach, the blood flow rate (cardiac output, \dot{Q}) is defined as:

$$\dot{Q} = \Delta P \times C, \quad (1)$$

or:

$$\dot{Q} = \Delta P / R, \quad (2)$$

where, ΔP is arterio-venous pressure difference, C is conductance and R is resistance.

¹Department of Biology, Portland State University, Portland, OR 97207, USA.

²Department of Biological Sciences, California State University East Bay, Hayward, CA 94542, USA.

*Author for correspondence (hillmans@pdx.edu)

List of symbols and abbreviations

C	vascular conductance
E–C coupling	excitation–contraction coupling
f_H	heart rate
\dot{Q}	blood flow rate (cardiac output)
R	resistance
RVM	relative ventricle mass
$\dot{V}_{O_2, \max}$	maximal rates of aerobic metabolism
V_S	stroke volume
ΔP	arterio-venous pressure difference

The capacity of the heart to do work over time (cardiac power) can be calculated as:

$$\text{Cardiac power} = \dot{Q} \times \Delta P = \dot{Q} \times (\dot{Q} \times R) = \dot{Q}^2 / C. \quad (3)$$

A second approach to quantify \dot{Q} involves characterizing the physical characteristics of the heart (heart rate f_H and stroke volume V_S) without considering the characteristics of the vasculature:

$$\dot{Q} = f_H \times V_S. \quad (4)$$

When the two approaches are measured together, a comprehensive understanding can be achieved of how each parameter contributes to increasing cardiac output \dot{Q} across the vertebrate lineage. We are unaware of any systematic attempt to organize power, work, pressure, conductance, heart rate and stroke volume data over a wide range of vertebrate cardiac outputs. Measurements of these variables from rest to exercise are the basis for this meta-analysis; unfortunately, there is a limited data set available for these measurements. There are extensive data available for certain mammals (humans, rats, dogs and horses) but very limited data for the other classes except teleost fishes. For example, we were unable to find any reptile data presenting simultaneous measurements of flow and pressure during exercise.

This meta-analysis of key cardiovascular differences between ectotherms and endotherms addresses the following questions:

- (1) what are the performance characteristics of the vertebrate heart at rest and during exercise?;
- (2) how do these characteristics vary between ectotherms and endotherms?;
- (3) what is the relative role of vasculature conductance in determining limitations to flow?; and
- (4) how does cardiac power input relate to aerobic power output during exercise?

RESULTS

Incorporating all measurements from rest to exercise, cardiac output was significantly ($P < 0.0001$) and linearly related to vascular conductance for endotherms and ectotherms (Fig. 1A, Fig. 2A). The slopes were significantly different and would require driving forces of 17.09 kPa for endotherms and 3.33 kPa for ectotherms (Fig. 1A, Fig. 2A). Given the apparent internal constancy of pressure from rest to activity, cardiac power was linearly related to flow rate for both endotherms ($R^2 = 0.97$, $P < 0.0001$; Fig. 1B) and ectotherms ($R^2 = 0.85$, $P < 0.0001$; Fig. 1B, Fig. 2B). The slope difference (16.1 versus 3.5 mJ ml^{-1}) reflects the mean arterial pressure difference between the endotherms and ectotherms (Fig. 1A, Fig. 2A). Cardiac stroke work was linearly related to stroke volume in both the endotherms ($R^2 = 0.76$, $P < 0.0001$, slope 15.8 mJ ml^{-1} ; Fig. 1C) and ectotherms ($R^2 = 0.62$, $P < 0.0001$, slope 2.4 mJ ml^{-1} ; Fig. 2C). The slope difference in these relationships reflects the inherent arterial pressure between the two groups. Cardiac power was significantly related to stroke work in both endotherms ($R^2 = 0.39$, $P = 0.0056$, slope 5.3 beats s^{-1} ; Fig. 1D) and ectotherms ($R^2 = 0.7$, $P < 0.0001$, slope 0.47 beats s^{-1} ; Fig. 2D).

Focusing now on only the values recorded during exercise for these species, better reflecting a limiting flow condition, the endotherms have significantly greater body mass-specific cardiac output ($P = 0.0013$; Fig. 3A) and greater heart rate ($P < 0.0001$; Fig. 3B) compared with the ectothermic values, but body mass-specific stroke volumes were not different ($P = 0.192$; Fig. 3C). The mean arterial pressure was greater for the endotherms ($P = 0.0039$; Fig. 3D), but vascular conductance was not different ($P = 0.274$; Fig. 3E) from the ectothermic values.

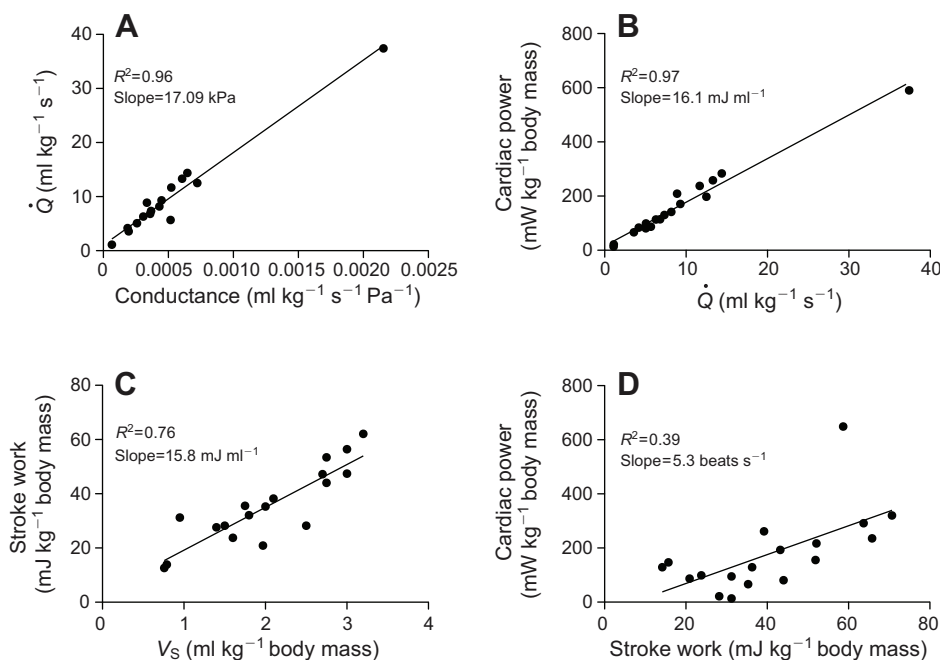


Fig. 1. Body mass-specific relationships for endotherms at rest and during exercise.

Data are from birds and mammals. (A) Cardiac output (\dot{Q}) and vascular conductance; (B) cardiac power and cardiac output; (C) stroke work and stroke volume (V_S); and (D) cardiac power and stroke work.

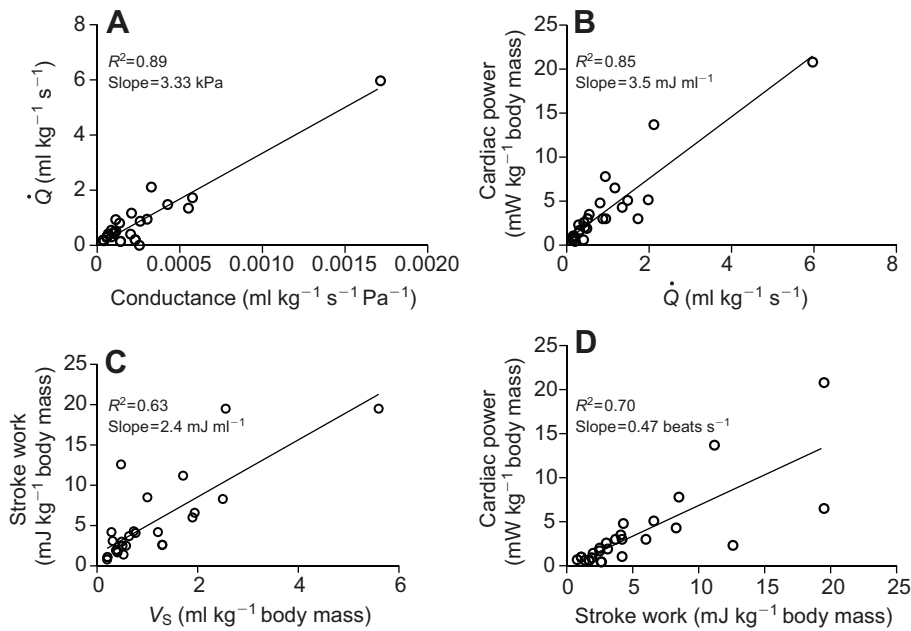


Fig. 2. The body mass-specific relationships for ectotherms at rest and during exercise. (A) Cardiac output \dot{Q} and vascular conductance; (B) cardiac power and cardiac output; (C) stroke work and stroke volume (V_S); and (D) cardiac power and stroke work.

Given the greater flows and pressures for the endotherms, the overall cardiac power was significantly greater ($P=0.0002$; Fig. 3F) compared with ectotherms.

Total flow rates during exercise were significantly linearly related to \dot{V}_{O_2} for the two groups combined ($R^2=0.91$, $P<0.0001$; Fig. 4A). Cardiac power was also significantly related to aerobic metabolic power for the two groups combined, with a slope of 158 mW of aerobic power output per mW of cardiac power input ($R^2=0.94$, $P<0.0001$; Fig. 4B). Ventricular mass-specific power was significantly greater in endotherms compared with ectotherms ($P<0.0001$). Body mass-specific stroke work was significantly greater ($P=0.0003$; Fig. 5A) in endotherms compared with ectotherms, while ventricle mass-specific stroke work was not different ($P=0.167$; Fig. 5B) between the groups. The relative ventricle mass was significantly greater ($P<0.0001$; Fig. 5C) in endotherms compared with ectotherms. Stroke volume was significantly linearly related to relative ventricle mass in both the endotherms ($R^2=0.57$, $P=0.0069$) and

ectotherms ($R^2=0.77$, $P=0.0008$), while the slope of the relationship was significantly steeper ($P=0.0062$; Fig. 5D) for the ectotherms (2.4 ml g^{-1} ventricle mass) compared with endotherms (0.56 ml g^{-1} ventricle mass).

DISCUSSION

The results of this meta-analysis clearly demonstrate that a major difference between ectotherms and endotherms is the large increase in blood flow rate. This can be attributed largely to a significantly greater heart rate in endotherms compared with ectotherms (Fig. 3B). Endotherms also maintain significantly higher levels of body mass-specific cardiac power (Fig. 3F) and cardiac stroke work (Fig. 5A), resulting in greater perfusion pressure to drive blood flow through the vascular network. These data suggest that a key step in the cardiovascular support of aerobic scope related to endothermy was faster heart rate, achieved by both elevated body temperature and higher rates of Ca^{2+} cycling (see below), and greater cardiac stroke work producing higher perfusion pressure to

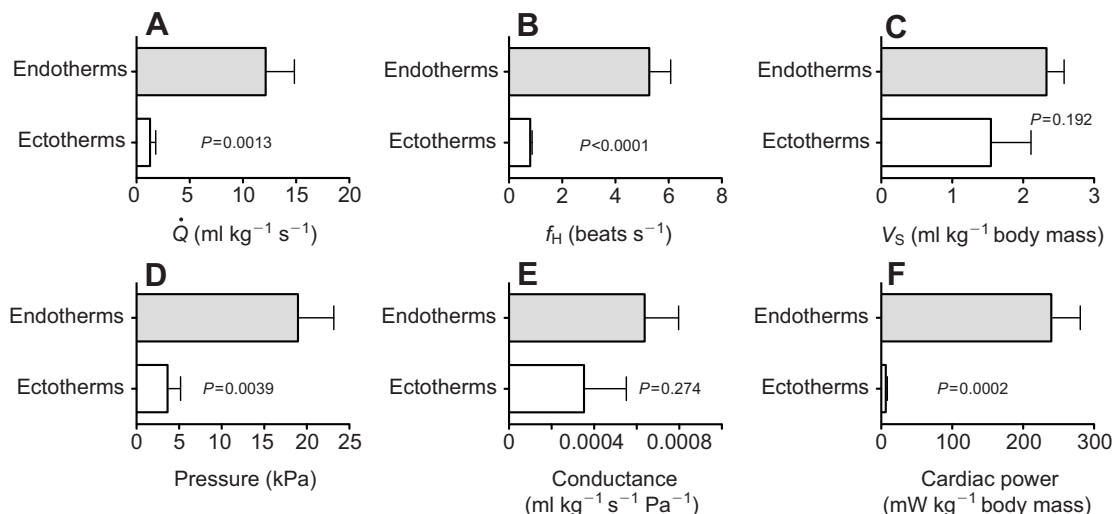


Fig. 3. Mean (and s.e.m.) body mass-specific values during exercise used in the meta-analysis for ectotherms and endotherms. (A) Cardiac output (\dot{Q}); (B) heart rate (f_H); (C) stroke volume (V_S); (D) mean arterial pressure; (E) vascular conductance; and (F) cardiac power required.

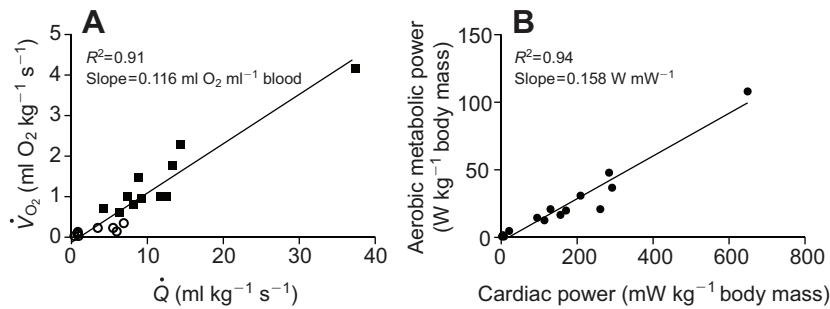


Fig. 4. The body mass-specific relationships during exercise for vertebrates. (A) Aerobic metabolism and cardiac output (\dot{Q}) (ectotherms, open circles; endotherms, squares). (B) Aerobic metabolic power and cardiac power.

support cardiovascular O_2 transport associated with endothermic metabolic rates.

Mitochondrial limits to $\dot{V}_{O_{2,max}}$ and evolution of endothermy

Previous work (Weibel et al., 2004; Weibel and Hoppeler, 2005) and a recent analysis (Clarke and Pörtner, 2010) have suggested that mitochondrial capacity limits maximal oxygen consumption and that proliferation of mitochondria was a key step in the evolution of endothermy. The mitochondrial limit hypothesis to maximal aerobic metabolism is not supported by several types of experimental evidence. First, if the number mitochondria limits maximal aerobic metabolism, this suggests that mitochondrial volume density in skeletal muscle fibres of ectotherms should be significantly less than that of endotherms. However, mitochondrial volume density of red muscle fibres of ectothermic fish (Johnston and Moon, 1981), amphibians (Marsh and Taigen, 1987) and reptiles (Schaeffer et al., 1996) can exceed the values found in endothermic birds and mammals. Consequently, if examples of greater mitochondrial capacity can be found from all ectothermic vertebrate classes, it falsifies a fundamental capacity difference from endotherms, and does not support the hypothesis that mitochondrial capacity was the key step in the evolution of endothermic metabolism.

Second, the mitochondrial hypothesis also suggests that there is no rate-limiting step in the O_2 delivery system to support $\dot{V}_{O_{2,max}}$ (Clarke and Pörtner, 2010) despite abundant evidence that

cardiovascular O_2 transport is the rate-limiting step to maximal O_2 transport across the vertebrate lineage (Hillman et al., 2013). The concept of symmorphosis (Weibel et al., 1991; Weibel, 2000) suggested there was no rate-limiting step to maximal O_2 transport because it was assumed that natural selection would eliminate excess capacity in a complex pathway such as O_2 transport (Weibel et al., 1991). The problem with the symmorphosis concept as applied to rate-limitation analysis is that it focuses solely on O_2 and ignores the role that these same transport processes play in eliminating CO_2 produced by the mitochondria (see Hillman et al., 2013).

Cardiovascular changes in ectotherms and endotherms

Vascular conductance was linearly related to total flow rate when all values from rest to exercise were considered for both groups (Fig. 1A, Fig. 2A). This can be viewed from two perspectives. First, some of the variation reflects inherent interspecific variation in the vasculature; that is, species with high cardiac outputs also have high conductances. The second consideration is that variation in cardiac output is coupled to variation in conductance. Both interspecific differences in conductance and cardiac output-induced variation in conductance have been documented for amphibians using an *in situ* perfusion technique (Kohl et al., 2013). Physiological control, via negative feedback control mediated by baroreceptors, increases conductance and permits increased flow, preventing pressure overload on the heart by

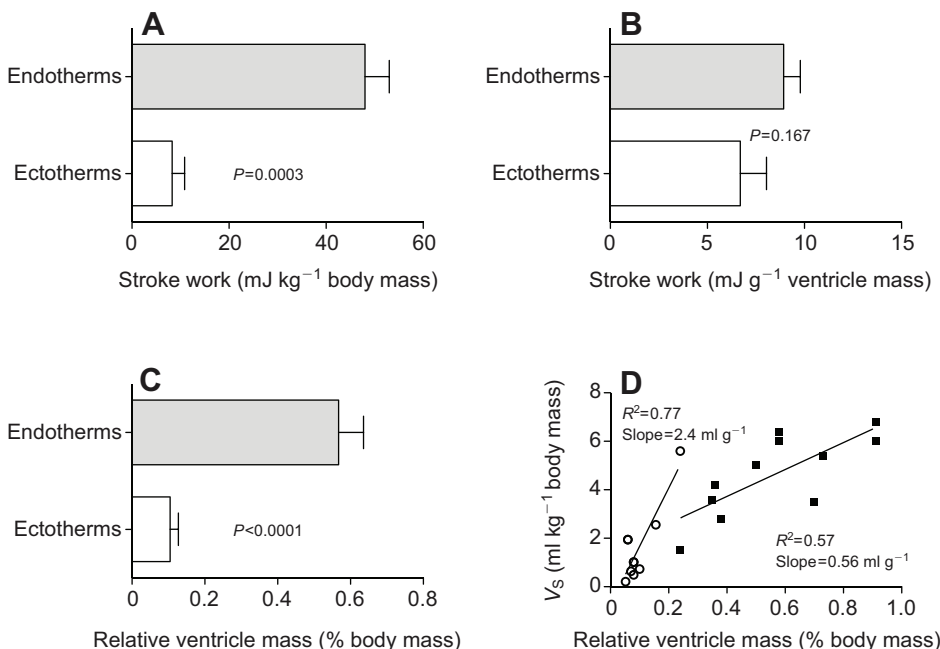


Fig. 5. Mean (and s.e.m.) exercise values for stroke work, relative ventricle mass and stroke volume for ectotherms and endotherms. (A) Body mass-specific stroke work; (B) ventricle mass-specific stroke work; (C) relative ventricle mass; and (D) the relationship between body mass-specific stroke volume (V_s) and relative ventricle mass (ectotherms, circles; endotherms, squares).

maintaining blood pressure. Increases in cardiac output also increase conductance by physically dilating resistance vessels, based on the increased volume added to the arterial vasculature. It is likely that the mechanisms matching conductance and flow are probably a combination of both passive physical effects and physiological effects mediated through negative feedback (Hillman et al., 2014). The difference between ectotherms and endotherms in the slope of the conductance–flow relationship can be attributed to the endotherms maintaining a higher pressure (17.1 kPa) compared with ectotherms (3.3 kPa). The apparent constancy of arterial pressure with variation in flow explains why cardiac power and stroke work are proportionally related to flow variables and to each other. Baroreceptor mediation would predict a relatively constant pressure from rest to exercise.

Considering only the data collected during exercise, the almost 10-fold greater flow rates of the endotherms compared with the ectotherms are physically explained by the 5.2 times greater arterial pressure (Fig. 3D) and 1.8 times greater vascular conductance (Fig. 3E) of the endotherms. The increased blood flow rates of the endotherms compared with the ectotherms is physiologically explained by a 6.6 times faster heart rate (Fig. 3B) and a 1.5 times greater stroke volume (Fig. 3C) of the endotherms. Consequently, the cardiac power difference of about 40-fold between the endotherms and ectotherms (Fig. 3F) is the combination of both flow rate (65%) and pressure differences (35%).

Metabolic rate during exercise was linearly related to total flow rate (Fig. 4A), consistent with a systemic O_2 transport limit to aerobic metabolism during exercise. The slope of the relationship was lower in ectotherms, but not significantly so ($P=0.079$), which is to be expected owing to the lower blood O_2 capacitance in ectotherms, the result of lower hematocrit (Pough, 1980). There is a consistent relationship between cardiac power input and aerobic metabolic power output (Fig. 4B) such that 158 mW (± 24 mW, 95% CI) of aerobic power output is achieved for every mW of cardiac power input. Thus, the metabolic cost of the working vertebrate heart during exercise appears to be only about 0.6% of the metabolic capacity that is aerobically supported. This value is lower than the mean value of 5.8% reported by Loiselle and Gibbs (1979) for resting papillary muscle *in vitro* from different species of mammals. Using their values for resting cardiac power and resting metabolic power gives a mean value of cardiac power representing 1.5% of the resting metabolism. If one assumes that exercise aerobic power output is 6–10 times that of basal aerobic power, the values for cardiac metabolic cost reported by Loiselle and Gibbs (1979) are comparable to the value we report (0.6%) for vertebrates.

The question then becomes one from a cardiac perspective: what explains the cardiac power difference that results from higher arterial pressures and heart rates between the endotherms and ectotherms? Body mass-specific stroke work was 5.8 times greater in the endotherms (Fig. 5A), while the ventricle mass-specific stroke work was equivalent between the groups (Fig. 5B). The ventricle mass-specific stroke work for both endotherms and ectotherms of 8–10 mJ g^{-1} is consistent with *in vitro* calorimetric measurements of mammalian cardiac papillary muscles when fully loaded (Loiselle and Gibbs, 1979) and toad cardiac muscle strips at external afterloads with the greatest mechanical efficiencies (Holroyd and Gibbs, 1993). The relative ventricle mass of the endotherms was 5.4 times greater than that of the ectotherms (Fig. 5C). The mean difference in ventricular mass between the ectotherms and endotherms was equivalent to the mean arterial

pressure difference (Fig. 1A, Fig. 2A). This indicates that body mass-specific power and stroke work differences are caused by greater ventricular muscle mass and not inherently stronger cardiac muscle. This is consistent with a comparison of the aerobic capacity of heart muscle from a reptile and mammal showing that the cardiac cost of delivering oxygen at the limits of aerobic capacity is similar in an ectotherm and endotherm (Conley et al., 1995). Within each group, increased ventricular mass was related to increased stroke volume (Fig. 5D), with stroke volume increasing 4.3 times faster with relative ventricle mass in ectotherms compared with endotherms. However, the resultant stroke volumes during exercise were not significantly different between the groups (Fig. 3C), indicating the increased RVM of the endotherms is physically responsible for generating the 5-fold greater arterial pressures in the endotherms. When one takes extreme values for RVM in any ectotherm (0.39 ml kg^{-1} body mass in *C. hamatus*; Tota et al., 1991), our equation predicts a stroke volume of 9.4 ml kg^{-1} body mass. This is consistent with measured stroke volumes of 10–11 ml kg^{-1} body mass (Tota et al., 1991). Extreme values for RVM in endotherms are approximately 1.6 ml kg^{-1} body mass (see Bishop, 1997), which would predict stroke volumes of 11 ml kg^{-1} body mass from our equation. It does not appear that extreme values for stroke volume differ between ectotherms and endotherms.

Do the increased heart rates between the endotherms and ectotherms relate to inherent increases in excitation–contraction (E–C) Ca^{2+} cycling or simply temperature effects on cardiac function in general (i.e. Q_{10})? The Q_{10} for the heart rate differences between the ectothermic (17°C) and endothermic (37°C) species summarized here was approximately 2.6. An additional factor to consider is that the ectothermic species had a significantly lower average body mass than the endothermic species. Because maximal heart rates decrease with increasing body mass in endotherms and ectotherms (Lillywhite et al., 1999; Bishop, 1997), it is necessary to normalize the data to equivalent body mass. Bishop (1997) presents regression equations for maximal heart rate and body mass in both mammals and birds. The equations predict exercise heart rates of between 477 and 488 beats min^{-1} for a 1 kg endotherm, the mean mass of the ectothermic species included in this analysis. The Q_{10} of this comparison is 3.2. The Q_{10} values for most ectothermic heart rates and maximal metabolic rates at the upper range of their tolerated temperatures are about 1.2–1.8 (Licht, 1965; Seymour, 1973; Carey, 1979; Overgaard et al., 2012); therefore, using an extreme Q_{10} of 2 would only account for about 42% of the heart rate for a 1 kg endotherm. Because these Q_{10} values do not approach the ectotherm-to-endotherm Q_{10} of 3.2, this indicates that, in conjunction with temperature, enhanced rates of E–C Ca^{2+} cycling are also necessary to explain the remaining 58% of the elevated heart rates of endotherms. A recent analysis indicates that the combination of both trans-sarcolemmal and sarcoplasmic reticulum Ca^{2+} to E–C coupling in cardiac muscle cells contributes to higher heart rates in both ectothermic and endothermic vertebrates (Shiels and Galli, 2014).

Given widespread interest in the evolution of endothermy from ectothermic ancestors (occurring at least twice in the vertebrate lineage), how does this meta-analysis influence current hypotheses? The results of this analysis suggest the increased cardiac power output and stroke work to support the aerobic power requirements of endotherms are achieved by increased rates of blood flow (65%), mainly as a result of increased heart

Table 1. Species, state, cardiac output, conductance, heart rate, cardiac power and stroke work used for the species in this meta-analysis

Species	State	\dot{Q} (ml kg ⁻¹ s ⁻¹)	C (ml kg ⁻¹ s ⁻¹ kPa ⁻¹)	f_H (beats s ⁻¹)	Cardiac power (mW kg ⁻¹ body mass)	Stroke work (mJ g ⁻¹ ventricle mass beat ⁻¹)
Ectotherms						
<i>T. semifasciata</i>	R	0.55	0.09	0.85	3.5	5.1
	E	0.94	0.11	0.92	4.4	10.6
<i>S. stellaris</i>	R	0.88	0.26	0.72	3	7
	E	1.48	0.43	0.77	5.1	11
<i>O. mykiss</i>	R	0.44	0.1	0.8	2	2.5
	E	0.81	0.14	1.12	4.8	4.3
<i>G. morhua</i>	R	0.31	0.06	0.72	1.4	2.5
	E	0.42	0.07	0.85	2.6	3.8
<i>P. borchgrevinki</i>	R	0.49	0.1	0.18	2.3	1.7
	E	1.17	0.21	0.33	6.5	12.5
<i>H. americanus</i>	R	0.31	0.09	0.62	1.9	4.4
	E	0.51	0.21	0.82	3.0	5.2
<i>A. australis</i>	R	0.19	0.04	0.9	1.0	2.1
	E	0.19	0.04	0.9	1.0	2.1
<i>P. bernachii</i>	R	0.29	0.08	0.25	1.06	4.0
<i>O. elongatus</i>	R	0.19	0.4	0.48	0.9	2.3
<i>R. marina</i>	R	0.95	0.3	0.5	3	2.6
	R	1.35	0.55	0.52	4.3	3.5
	E	6	1.72	1.07	20.8	8.1
	R	2.63	0.43	0.85	16.1	8.2
E	8.4	1.72	1.71	40.8	10.3	
Endotherms						
<i>C. livia</i>	R	5.05	0.36	1.83	81	4.8
	E	37.4	2.16	11.05	649	6.4
	R	5.68	0.4	3	87	2.3
	E	12.5	0.72	4.17	217	8.7
<i>A. platyrhynchos</i>	R	5.07	0.28	4.17	99	3.4
	E	11.7	0.52	6.67	261	5.6
<i>D. novaehollandiae</i>	R	1.13	0.07	0.77	22	3.9
	E	8.2	0.43	3	156	7.1
<i>H. sapiens</i>	R	1.4	0.1	1.17	17.1	3.4
	E	4.2	0.19	3.03	94.4	8.2
<i>C. familiaris</i>	R	3.6	0.2	1.88	66.4	6.1
	E	13.3	0.61	4.58	291.6	11
	E	14.4	0.73	4.57	284	12.1
<i>R. norvegicus</i>	E	6.8	0.41	9.17	130	6.5
<i>C. hircus</i>	E	9.3	0.51	4.47	171	12
<i>B. taurus</i>	E	6.3	0.35	3.55	114	10.4
<i>E. caballus</i>	E	8.9	0.58	3.58	209	13.2

R, rest; E, exercise; \dot{Q} , cardiac output; C, conductance; f_H , heart rate.

See Materials and methods, 'Species', for full species names and sources of data.

rate, and increased arterial pressure (35%), achieved via increased ventricular muscle mass. Our results indicate that a key step in the support of endothermy was the greatly enhanced ability of the cardiovascular system to deliver oxygen, which accounted for the approximately 10-fold increase in aerobic scope between endotherms and ectotherms.

MATERIALS AND METHODS

Species

The species used in this meta-analysis were: Chondrichthyes – *Triakis semifasciata* (Lai et al., 1989, 1990), *Scyliorhinus stellaris* (Piiper et al., 1977); Osteichthyes – *Hemirhamphus americanus* (Axelsson et al., 1989), *Anguilla australis* (Davie and Forster, 1980), *Gadus morhua* (Axelsson, 1988; Axelsson and Nilsson, 1986; Gallagher, 1994), *Oncorhynchus mykiss* (Kiceniuk and Jones, 1977), *Pogonias cromis* and *P. borchgrevinki* (Axelsson et al., 1992), *Ophiodon elongatus* (Farrell, 1982); Amphibia – *Rhinella marina* (West and Smits, 1994; Withers et al., 1988; Hedrick et al., 1999); Aves – *Columba livia* (Grubb, 1982; Peters et al., 2005), *Anas platyrhynchos* (Grubb, 1982), *Dromiceius novaehollandiae* (Grubb et al., 1983); Mammalia – *Homo sapiens* (Brooks et al., 1996), *Canis familiaris* (Stray-Gundersen et al., 1986; Karas et al., 1987), *Bos taurus* (Karas et al.,

1987), *Equus caballus* (Karas et al., 1987), *Capra hircus* (Karas et al., 1987) and *Rattus norvegicus* (Henderson et al., 2002). Data for these species are given in Table 1.

Cardiac power calculations

The specific expressions are expressed relative to body mass (kg) or relative to total ventricle mass (g) and assume an isometric relationship to mass.

For endotherms, as the pulmonary and systemic flows are in series and usually only systemic flows and arterial pressures (P_a) are reported:

$$\text{Power} = 1.1 \times (P_a \times \text{systemic } \dot{Q}), \quad (5)$$

where power is in mW, P_a is in kPa and systemic cardiac output (\dot{Q}) is in ml kg⁻¹ s⁻¹. The term 1.1 accounts for total ventricular power (both right and left side), as the right side pulmonary P_a is 9–14% that of the systemic vasculature at rest and during exercise (Grubb, 1982; Stray-Gundersen et al., 1986).

For ectotherms:

$$\text{Power} = P_a \times \text{total } \dot{Q}. \quad (6)$$

Stroke work calculations

Stroke work (mJ) was expressed relative to body mass (kg) or relative to total ventricle mass (g) and assumed an isometric relationship to mass:

$$\text{Stroke work} = \text{power} \times f_H^{-1},$$

where f_H^{-1} is in s beat^{-1} .

Metabolic energetic equivalence

One ml of O_2 consumed was assumed to be 20.9 J. Aerobic metabolic rates during activity were taken directly from the references for the cardiovascular measurements. If no data were reported, they were not included in the analyses.

Statistics

Least-square linear regression techniques were used to determine relationships. Endotherm–ectotherm comparisons were made with Prism version 5 (GraphPad Software, La Jolla, CA, USA) using two-tailed *t*-tests with significance being $P < 0.05$.

Acknowledgements

We wish to thank Z. Kohl, G. Kluthe and Drs G. Spolek and G. Brodowicz for discussions in the development of this work.

Competing interests

The authors declare no competing or financial interests.

Author contributions

The authors contributed equally to the meta-analysis, manuscript and interpretation of the findings.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

- Andersen, P. and Saltin, B. (1985). Maximal perfusion of skeletal muscle in man. *J. Physiol.* **366**, 233-249.
- Axelsson, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod *Gadus morhua* at rest and non-exhaustive exercise. *J. Exp. Biol.* **137**, 287-303.
- Axelsson, M. and Nilsson, S. (1986). Blood pressure regulation during exercise in the Atlantic cod *Gadus morhua*. *J. Exp. Biol.* **126**, 225-236.
- Axelsson, M., Driedzic, W. R., Farrell, A. P. and Nilsson, S. (1989). Regulation of cardiac output and gut blood flow in the sea raven, *Hemirhamphus americanus*. *Fish Physiol. Biochem.* **6**, 315-326.
- Axelsson, M., Davison, W., Forster, M. E. and Farrell, A. P. (1992). Cardiovascular responses of the red-blooded Antarctic fishes, *Pagothenia bemaichii* and *P. borchgrevinkii*. *J. Exp. Biol.* **167**, 179-201.
- Bennett, A. F. and Ruben, J. A. (1979). Endothermy and activity in vertebrates. *Science* **206**, 649-654.
- 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. Lond. B Biol. Sci.* **352**, 447-456.
- Bishop, C. M. (1999). The maximum oxygen consumption and aerobic scope of birds and mammals: getting to the heart of the matter. *Proc. R. Soc. Lond. B Biol. Sci.* **266**, 2275-2281.
- Brooks, G. A., Fahey, T. D. and White, T. P. (1996). *Exercise Physiology: Human Bioenergetics and Its Applications*. Mt. View, USA: Mayfield Publishing.
- Carey, C. (1979). Aerobic and anaerobic energy expenditure during rest and activity in montane *Bufo b. boreas* and *Rana pipiens*. *Oecologia* **39**, 213-228.
- Clarke, A. and Pörtner, H.-O. (2010). Temperature, metabolic power and the evolution of endothermy. *Biol. Rev.* **85**, 703-727.
- Conley, K. E., Christian, K. A., Hoppeler, H. and Weibel, E. R. (1995). Heart mitochondrial properties and aerobic capacity are similarly related in a mammal and a reptile. *J. Exp. Biol.* **198**, 739-746.
- Cowles, R. B. (1958). Possible origin of dermal temperature regulation. *Evolution* **12**, 347-357.
- Davie, P. S. and Forster, M. E. (1980). Cardiovascular responses to swimming in eels. *Comp. Biochem. Physiol. A Physiol.* **67**, 367-373.
- Dawson, T. J., Webster, K. N., Lee, E. and Buttemer, W. A. (2013). High muscle mitochondrial volume and aerobic capacity in a small marsupial (*Sminthopsis crassicaudata*) reveals flexible links between energy-use levels in mammals. *J. Exp. Biol.* **216**, 1330-1337.
- di Prampero, P. E. (2003). Factors limiting maximal performance in humans. *Eur. J. Appl. Physiol.* **90**, 420-429.
- Farrell, A. P. (1982). Cardiovascular changes in the unanaesthetized lingcod (*Ophiodon elongatus*) during short-term, progressive hypoxia and spontaneous activity. *Can. J. Zool.* **60**, 933-941.
- Frappell, P., Schultz, T. and Christian, K. (2002). Oxygen transfer during aerobic exercise in a varanid lizard *Varanus mertensi* is limited by the circulation. *J. Exp. Biol.* **205**, 2725-2736.
- Gallaugh, P. E. (1994). The role of hematocrit in oxygen transport in swimming salmonid fishes, pp. 279. PhD thesis, Department of Biological Sciences, Simon Fraser University, Burnaby.
- Gallaugh, P. E., Thorarensen, H., Kiessling, A. and Farrell, A. P. (2001). Effects of high intensity exercise training on cardiovascular function, oxygen uptake, internal oxygen transport and osmotic balance in chinook salmon (*Oncorhynchus tshawytscha*) during critical speed swimming. *J. Exp. Biol.* **204**, 2861-2872.
- Grubb, B. A. (1982). Cardiac output and stroke volume in exercising ducks and pigeons. *J. Appl. Physiol.* **53**, 207-211.
- Grubb, B. A., Jorgensen, D. D. and Conner, M. (1983). Cardiovascular changes in the exercising emu. *J. Exp. Biol.* **104**, 193-201.
- Hedrick, M. S., Palioca, W. B. and Hillman, S. S. (1999). Effects of temperature and physical activity on blood flow shunts and intracardiac mixing in the toad *Bufo marinus*. *Physiol. Biochem. Zool.* **72**, 509-519.
- Henderson, K. K., Wagner, H., Favret, F., Britton, S. L., Koch, G., Wagner, P. D. and Gonzales, N. C. (2002). Determinants of maximal O_2 uptake in rats selectively bred for endurance running capacity. *J. Appl. Physiol.* **93**, 1265-1274.
- Hillman, S. S., Hancock, T. V. and Hedrick, M. S. (2013). A comparative meta-analysis of maximal aerobic metabolism of vertebrates: implications for respiratory and cardiovascular limits to gas exchange. *J. Comp. Physiol. B.* **183**, 167-179.
- Hillman, S. S., Hedrick, M. S. and Kohl, Z. F. (2014). Net cardiac shunts in anuran amphibians: physiology or physics? *J. Exp. Biol.* **217**, 2844-2847.
- Holroyd, S. M. and Gibbs, C. L. (1993). The energetics of shortening amphibian cardiac muscle. *Pflügers Arch.* **424**, 84-90.
- Johnston, I. A. and Moon, T. W. (1981). Fine structure and metabolism of multiply innervated fast muscle fibres in teleost fish. *Cell Tissue Res.* **219**, 93-109.
- Karas, R. H., Taylor, C. R., Rösler, K. and Hoppeler, H. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand: V. Limits to oxygen transport by the circulation. *Respir. Physiol.* **69**, 65-79.
- Kiceniuk, J. W. and Jones, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. Exp. Biol.* **69**, 247-260.
- Kohl, Z. F., Hedrick, M. S. and Hillman, S. S. (2013). Separating the contributions of vascular anatomy and blood viscosity to peripheral resistance and the physiological implications of interspecific resistance variation in amphibians. *J. Comp. Physiol. B* **183**, 921-932.
- Lai, N. C., Graham, J. B., Lowell, W. R. and Shebat, R. (1989). Elevated pericardial pressure and cardiac output in the leopard shark, *Triakis semifasciata*, during exercise: the role of the pericardioperitoneal canal. *J. Exp. Biol.* **147**, 263-277.
- Lai, N. C., Graham, J. B. and Burnett, L. (1990). Blood respiratory properties and the effect of swimming on blood gas transport in the leopard shark *Triakis semifasciata*. *J. Exp. Biol.* **151**, 161-173.
- Licht, P. (1965). Effects of temperature on heart rates of lizards during rest and activity. *Physiol. Zool.* **38**, 129-137.
- Lillywhite, H. B., Zippel, K. C. and Farrell, A. P. (1999). Resting and maximal heart rates in ectothermic vertebrates. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **124**, 369-382.
- Loiselle, D. S. and Gibbs, C. L. (1979). Species differences in cardiac energetics. *Am. J. Physiol.* **237**, H90-H98.
- Marsh, R. L. and Taigen, T. L. (1987). Properties enhancing aerobic capacity of calling muscles in gray tree frogs *Hyla versicolor*. *Am. J. Physiol.* **252**, R786-R793.
- McNab, B. K. (1978). The evolution of endothermy in the phylogeny of mammals. *Am. Nat.* **112**, 1-21.
- Overgaard, J., Andersen, J. L., Findsen, A., Pedersen, P. B. M., Hansen, K., Ozolina, K. and Wang, T. (2012). Aerobic scope and cardiovascular oxygen transport is not compromised at high temperatures in the toad *Rhinella marina*. *J. Exp. Biol.* **215**, 3519-3526.
- Peters, G. W., Steiner, D. A., Rigoni, J. A., Mascilli, A. D., Schnepf, R. W. and Thomas, S. P. (2005). Cardiorespiratory adjustments of homing pigeons to steady wind tunnel flight. *J. Exp. Biol.* **208**, 3109-3120.
- Piiper, J., Meyer, M., Worth, H. and Willmer, H. (1977). Respiration and circulation during swimming activity in the dogfish *Scyliorhinus stellaris*. *Respir. Physiol.* **30**, 221-239.
- Pough, F. H. (1980). The advantages of ectothermy for tetrapods. *Am. Nat.* **115**, 92-112.
- Powers, S. K., Lawler, J., Dempsey, J. A., Dodd, S. and Landry, G. (1989). Effects of incomplete pulmonary gas exchange on $\text{VO}_{2\text{max}}$. *J. Appl. Physiol.* **66**, 2491-2495.
- Schaeffer, P. J., Conley, K. E. and Lindstedt, S. L. (1996). Structural correlates of speed and endurance in skeletal muscle: the rattlesnake shaker muscle. *J. Exp. Biol.* **199**, 351-358.

- Seymour, R. S.** (1973). Physiological correlates of forced activity and burrowing in the spadefoot toad, *Scaphiopus hammondi*. *Copeia* **1973**, 103-115.
- Shiels, H. A. and Galli, G. L. J.** (2014). The sarcoplasmic reticulum and the evolution of the vertebrate heart. *Physiology* **29**, 456-469.
- Stray-Gundersen, J., Musch, T. I., Haidet, G. C., Swain, D. P., Ordway, G. A. and Mitchell, J. H.** (1986). The effect of pericardiectomy on maximal oxygen consumption and maximal cardiac output in untrained dogs. *Circ. Res.* **58**, 523-530.
- Tota, B., Acierno, R. and Agnisola, C.** (1991). Mechanical performance of the isolated and perfused heart of the haemoglobinless Antarctic icefish *Chionodraco hamatus* (Lonnberg): effects of loading conditions and temperature. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **332**, 191-198.
- Weibel, E. R.** (2000). *Symmorphosis: On Form and Function in Shaping Life*. Cambridge, MA: Harvard University Press.
- Weibel, E. R. and Hoppeler, H.** (2005). Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. *J. Exp. Biol.* **208**, 1635-1644.
- Weibel, E. R., Taylor, C. R. and Hoppeler, H.** (1991). The concept of symmorphosis: a testable hypothesis of structure-function relationship. *Proc. Natl. Acad. Sci. USA* **88**, 10357-10361.
- Weibel, E. R., Bacalalupe, L. D., Schmitt, B. and Hoppeler, H.** (2004). Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. *Respir. Physiol. Neurobiol.* **140**, 115-132.
- West, N. H. and Smits, A. W.** (1994). Cardiac output in conscious toads (*Bufo marinus*). *J. Exp. Biol.* **186**, 315-323.
- Withers, P. C. and Hillman, S. S.** (1988). A steady-state model of maximal oxygen and carbon dioxide transport in anuran amphibians. *J. Appl. Physiol.* **64**, 860-868.
- Withers, P. C., Hillman, S. S., Simmons, L. A. and Zygmunt, A. C.** (1988). Cardiovascular adjustments to enforced activity in the anuran amphibian, *Bufo marinus*. *Comp. Biochem. Physiol.* **89**, 45-49.