The Journal of Experimental Biology 207, 2305-2311
Published by The Company of Biologists 2004
doi:10.1242/jeb.01017

Respiration rate of hepatocytes varies with body mass in birds

Paul L. Else[1,*], M. D. Brand[3], N. Turner[1] and A. J. Hulbert[2]

[1]*Department of Biomedical Science,* [2]*Department of Biological Science, Metabolic Research Centre, University of Wollongong, Wollongong, NSW 2522, Australia* and [3]*MRC-Dunn Human Nutrition Unit, Hills Road, Cambridge CB2 2XY, UK*

*Author for correspondence (e-mail: pelse@uow.edu.au)

Accepted 7 April 2004

Summary

Hepatocytes were isolated from eight species of birds ranging from 13 g zebra finches to 35 kg emus. This represents a 2800-fold range of body mass (M_b). Liver mass (g) was allometrically related to species body mass by the equation: liver mass=19.6×$M_b^{0.91}$. There was a significant allometric decline in hepatocyte respiration rate (HRR; nmol O_2 mg^{-1} dry mass min^{-1}) with species body mass (kg) described by the relationship: HRR=5.27×$M_b^{-0.10}$. The proportions of hepatocyte oxygen consumption devoted to (i) mitochondrial ATP production, (ii) mitochondrial proton leak and (iii) non-mitochondrial processes were estimated by using excess amounts of appropriate inhibitors. It was found that although hepatocyte respiration rate varied with body mass in birds, these processes constitute a relatively constant proportion of hepatocyte metabolic rate irrespective of the size of the bird species. The respective percentages were 54%, 21% and 25%. The portion of hepatocyte respiration devoted to ATP production for use by the sodium pump was estimated and found to be a relatively constant 24% of hepatocyte respiration and 45% of mitochondrial ATP production in different-sized bird species. These results are discussed in the context of competing theories to explain the metabolism–body size allometry, and are found to support the 'allometric cascade' model.

Key words: allometry, body mass, metabolism, proton leak, sodium pumping, bird, hepatocyte.

Introduction

It was obvious to Sarrus and Rameaux in 1838 (cited by Brody, 1945) that if species differing greatly in body size were to maintain the same body temperature, their heat production could not be directly proportional to their body mass. Bigger species must have a lower mass-specific rate of metabolism than smaller species. They suggested instead that heat production must be proportional to the surface area of the animal, thus proposing the 'surface law' of metabolism. Since that time, many studies have shown that the resting metabolic rate of different-sized species varies with a power function of body mass (M_b), but that it is not the power function of mass expected if metabolic rate were proportional to surface area (i.e. $M_b^{2/3}$, assuming different-sized species are approximately the same shape and specific density). Rather, resting metabolic rate is proportional to a power function of mass between surface area proportionality and direct proportionality to mass (i.e. between $M_b^{0.67}$ and $M_b^{1.0}$). The actual exponent of this power function has varied between studies but the two earliest studies of mammals (Kleiber, 1932; Brody and Procter, 1932) independently obtained allometric exponents of 0.73–0.74 for this relationship. The average exponent for intra-specific studies is approximately 0.72, whilst for inter-specific studies the average is about 0.76 (see Withers, 1992). The similarity of these values to 3/4 has, over the years, led to many proposed explanations, some of which were based on various physical theories whilst others were more empirically based. These two very different approaches to explaining this long-known allometric relationship between metabolic rate and body size are evident in the recent clash between the fractal-based model of West et al. (1997, 2002) and the allometric cascade model of Darveau et al. (2002). The difference between these two explanations also emphasises two different perspectives as to whether the rate of metabolism is primarily determined by the 'supply' or 'demand' aspects of an animal's metabolism. The allometric scaling model of West and colleagues, based on fractal-like distribution networks, predicts that '*the metabolic power of a cultured mammalian cell should be the same, independent of the mammal of origin, whether from shrews or whales*' (p. 2477 of West et al., 2002). The argument between these two groups over the basis of the allometric relationship of metabolism to body mass has centred on data for mammals.

Birds have evolved endothermy independently of mammals. In this study we report an allometric analysis of the respiration rate of hepatocytes isolated from eight bird species ranging from 13 g zebra finches to 35 kg emus. This represents a 2800-fold variation in body mass. We estimated, by the use of

inhibitors, the quantitative importance of four subcellular processes to the *in vitro* oxygen consumption of these hepatocytes. These processes are: total mitochondrial production of ATP, mitochondrial ATP production devoted to sodium pumping, mitochondrial proton leak and non-mitochondrial oxygen consumption. We also compare our findings for birds with those previously reported for hepatocytes isolated from different-sized mammals (Porter and Brand, 1995).

Materials and methods

All chemicals were from Sigma-Aldrich (Castle Hill, NSW, Australia) except for collagenase (Type 1; 307 U mg^{-1}) enzyme, which was from Worthington Chemicals (Freehold, NJ, USA). All experiments were approved by the University of Wollongong Animal Experimentation Ethics Committee.

Emus (*Dromaius novaehollandiae* Latham) were purchased from Marayong Park Emu Farm (Falls Creek, NSW, Australia). Zebra finches (*Taeniopygia guttata* Vieillot), domestic ducks (*Anas platyrhynchos* L.) and domestic geese (*Anser anser* L.) were purchased from local pet shops or the Narellan Aviary Bird Auction (NSW, Australia). Feral pigeons (rock dove *Columba livia* Gmelin) were from a pigeon breeder (T. Cooper, Corrimal, NSW, Australia). House sparrows (*Passer domesticus* L.), starlings (*Sturnus vulgaris* L.) and pied currawongs (*Strepera graculina* Shaw) were trapped in or near Wollongong, NSW, Australia. All birds were killed by anaesthetic overdose (sodium pentobarbitone, 100 mg kg^{-1} body mass; intraperitoneal, except for emus where injection was intrajugular) within a few days of purchase or capture. When birds were kept in captivity for a short period before sacrifice, they were provided with access to water and food *ad libitum* (mixed bird seed for finches and sparrows and a commercial mixture of pellets and seed for the ducks and geese). The diet of birds before their purchase/capture was unknown. The birds used in this study are the same birds for which other data has been previously reported (see Hulbert et al., 2002b; Brand et al., 2003).

Fresh liver tissue was rapidly removed from each bird and a piece of liver from the end of the major liver lobe was removed for hepatocyte preparation; the remainder was used for isolation of liver mitochondria (see Brand et al., 2003). In the case of zebra finches and sparrows, whole liver lobes were used for the preparation of hepatocytes. For hepatocyte isolation the cut section was trimmed to reveal the common major artery and cannulated. Blood was cleared using ice-cold pre-wash medium, containing (in mmol l^{-1}) 10 Hepes, 25 $NaHCO_3$, 125 NaCl, 3.4 KCl, 1.1 KH_2PO_4, 1.2 $MgSO_4$ and 11.1 glucose, 0.2% bovine serum albumin and 0.001% Phenol Red, pH 7.2, and gassed with 95% O_2, 5% CO_2, either directly from the perfusion apparatus or from a syringe passed into the cannulated artery, with blood and medium exiting *via* veins cut across the liver section. Liver cells were isolated by performing 5–10 min of pre-wash perfusion (at 25°C) with no recirculation followed by 20–30 min of recirculated collagenase perfusion medium (composition as pre-wash medium with collagenase added at 1 g l^{-1} and 1 mmol l^{-1} $CaCl_2$) at a flow rate of 2 ml min^{-1} (for small bird species) to 30 ml min^{-1} (for large bird species). The liver capsule of undamaged perfused sections of the liver was teased away and the loose liver cells were gently shaken out into pre-wash medium. Isolated cells were filtered through 250 μm nylon gauze, centrifuged twice for 4 min at 100 ***g*** and resuspended in suspension medium (as for pre-wash but containing 1 mmol l^{-1} $CaCl_2$) and stored on ice. Viabilities were assessed by Trypan Blue exclusion and varied with the body mass of the species. Preparations from emus, geese and ducks averaged 92%, 93% and 97% viability, respectively. Pigeon and currawong preparations both had an average viability of 89%, whilst hepatocyte preparations from starlings, sparrows and zebra finches averaged viabilities of 73%, 72% and 80%, respectively. The microscopic appearance of all isolated cell preparations showed no obvious blebbing.

Oxygen consumption was measured using a Strathkelvin (Glasgow, UK) oxygen electrode connected to a Strathkelvin 781 oxygen meter with electrode output coupled to a ADI Instruments (Sydney, Australia) PowerLab 400 data acquisition system coupled to a Macintosh powerbook. Oxygen consumption measurements were made between 39–40°C, representing an intermediate temperature between the normal body temperature of the passerine and non-passerine species examined. Cells were added to the oxygen electrode (final protein concentrations between 0.5–2.0 mg ml^{-1}) and oxygen consumption measured for 5–10 min following thermal equilibration. Calculations of oxygen consumption rates were corrected for the viability of that preparation. Following measurement of normal oxygen consumption rate, specific inhibitors were added sequentially to block components of metabolism. Oligomycin, a specific inhibitor of mitochondrial ATP synthase, was added stepwise (at 0.001 mg ml^{-1}) until the rate of oxygen consumption failed to decrease in response to further additions (usually at 0.002–0.004 mg ml^{-1} final concentration). The decrease in respiration following oligomycin addition was thus used to measure respiration devoted to ATP production. Myxothiazol, a specific inhibitor of mitochondrial oxygen consumption, was added stepwise (at 0.01 mg ml^{-1}) until oxygen consumption failed to decrease further in response to further additions (usually at 0.02–0.03 mg ml^{-1} final concentration). This further decrease following maximal myxothiazol inhibition was used to estimate respiration associated with proton leak (rate of respiration with oligomycin minus rate with oligomycin plus myxothiazol). In a number of preparations KCN was also added as a final inhibitor (to a final concentration of 5 mmol l^{-1}) to check the effectiveness of myxothiazol. Oxygen consumption of nonmitochondrial respiration was insensitive to myxothiazol. Sodium-pump-associated respiration was determined separately by incubation in the presence of ouabain (a specific inhibitor of the sodium pump) at 10^{-3} mol l^{-1} final concentration. The respiration rates of some hepatocyte preparations from a number of species were measured until all oxygen was depleted from the system. These preparations

showed constant rates of respiration for periods of up to 45 min over the full scale of oxygen partial pressures.

Dry mass of cells was determined by the difference in mass (to ±0.01 mg) between Eppendorf tubes with and without cells before and after drying, with correction for incubation solution salts. The drying process involved placing 0.2 ml of cell suspension (or incubation medium) in preweighed (dry) 2.5 ml Eppendorf tubes into an oven at 50°C for 5 days, followed by transfer to a vacuum-sealed desiccator with dry silica gel for 2 weeks at room temperature. Protein content was measured by the Lowry method using bovine serum albumin as a standard. Cell density was measured using a haemocytometer.

All figures were constructed using Kaleidagraph version 3.51 (Synergy Software, Reading, PA, USA) and statistics determined using JMP version 3 (SAS Institute, Cary, NC, USA).

Results

The bird species examined were chosen to span as wide a range of body masses as practicable and are listed in Table 1 together with their body mass M_b and basal metabolic rate (BMR). Fig. 1 presents an allometric plot of the basal metabolic rates (ml O_2 g^{-1} h^{-1}) of these particular species against their body mass M_b (kg). The four smallest species were all passerines whilst the four largest species were non-passerine birds. Because many passerines have been reported to have higher metabolic rates than similar-sized non-passerines (Lasiewski and Dawson, 1967), this difference between large and small species results in an allometric relationship for BMR=0.71×$M_b^{-0.36}$, which is steeper than that previously reported for passerine and non-passerine birds separately (Lasiewski and Dawson, 1967).

The liver represented a smaller percentage of total body mass as the size of the species increased. In zebra finches the liver averaged 2.71% of body mass compared to 1.87% in emus. This trend resulted in an allometric relationship of liver mass to body mass with an exponent of 0.91 (Fig. 2). which is similar to the situation in mammals where liver mass is related to the 0.87 power of body mass (see Hulbert and Else, 2000).

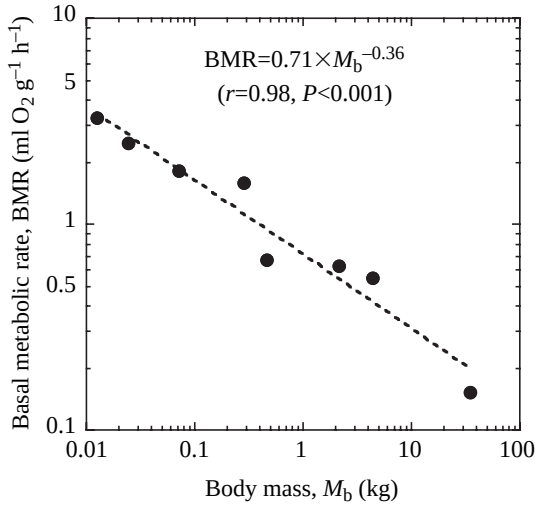

Fig. 1. The allometric relationship between body mass M_b (kg) and basal metabolic rate (BMR) for the bird species used in the current study. See Table 1 for names of individual species.

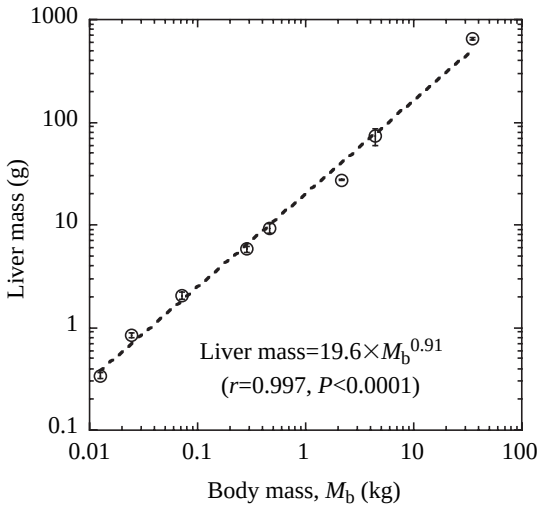

Fig. 2. The allometric relationship between liver mass (g) and body mass M_b (kg) of different-sized birds. Values are means ± S.E.M.; N=4 for all bird species.

Table 1. *Body mass and standard metabolic rate of the eight species of birds used in the present study*

Species		Body mass[a] (g)	Basal metabolic rate (ml O_2 g^{-1} h^{-1})[b]
Zebra finch	*Taeniopygia guttata*	12.6±1.0 (4)	3.28
House sparrow	*Passer domesticus*	24.6±0.7 (4)	2.46
Starling	*Sturnus vulgaris*	72.7±2.5 (4)	2.31
Pied currawong	*Strepera graculina*	283±19 (4)	1.59
Pigeon	*Columba livia*	462±35 (4)	0.667
Duck	*Anas platyrhynchos*	2178±61 (4)	0.626
Goose	*Anser anser*	4487±341 (4)	0.547
Emu	*Dromaius novaehollandiae*	34975±745 (4)	0.152

[a]Values are means ± S.E.M.; N (in parentheses) = total number of birds used per species.
[b]Basal metabolic rates from table 1 in Hulbert et al. (2002b).

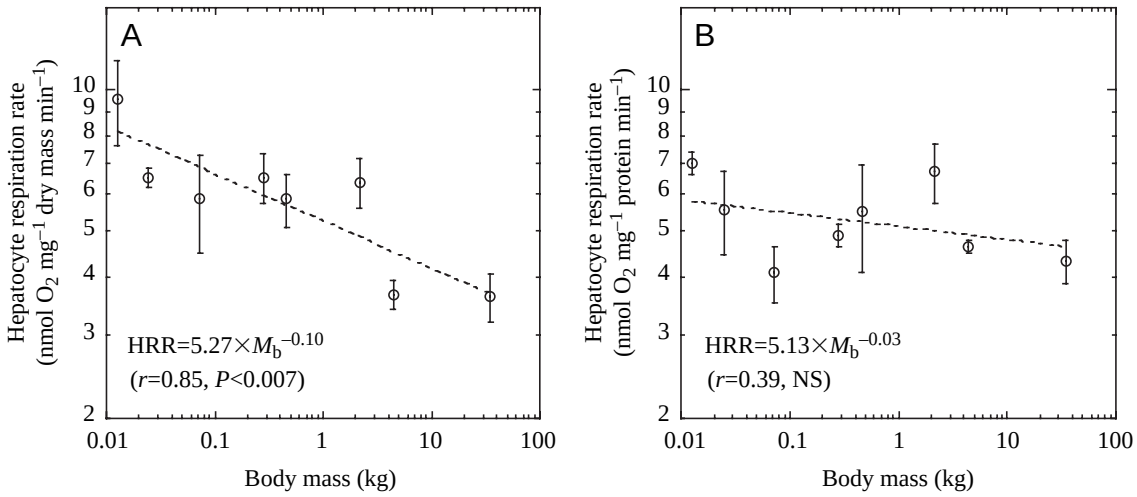

Fig. 3. The allometric relationship between respiration rate of isolated hepatocytes (HRR) and body mass M_b (kg) of different-sized birds. (A) HRR expressed per mg dry mass; (B) HRR expressed per mg protein. Values are means ±S.E.M. N=4 for all bird species except sparrows in A (N=2).

The respiration rate of hepatocytes isolated from the different-sized bird species, expressed relative to hepatocyte dry mass and hepatocyte protein content, are presented in Fig. 3. There was a statistically significant allometric decline in hepatocyte respiration with increasing body mass when expressed relative to dry mass but not when expressed relative to hepatocyte protein content. The allometric relationship between hepatocyte respiration rate (HRR; nmol O_2 mg^{-1} dry mass min^{-1}) and body mass M_b (kg) is HRR=5.27×$M_b^{-0.10}$ (P<0.001). The allometric exponent was –0.10 (95% confidence limits –0.04 to –0.16), which means that the respiration rates of hepatocytes from birds the size of zebra finches are approximately 2.2 times those of hepatocytes from emu-sized birds. The allometric relationship for respiration rate relative to hepatocyte protein content had a slope of –0.03, which was not statistically different from zero because the amount of hepatocyte protein relative to hepatocyte dry mass varied with species size. For example, protein represented an average 93.5% of dry mass in zebra finch hepatocytes but only 89.0% of dry mass in emu hepatocytes.

Measurement of both the cell density and protein content of each hepatocyte preparation allowed calculation of the protein content of the average hepatocyte for each preparation. This data is presented in Fig. 4 and shows that, on average, hepatocytes from smaller birds contain more protein per liver cell. Although the slope of this allometric relationship is not significantly different from zero, the equation indicates that whereas hepatocytes from zebra finches average 0.33 ng protein $cell^{-1}$, those from emus average 0.19 ng protein $cell^{-1}$. The reason for the variation in cell protein content is not known. Cell size was not measured in the current study and thus it is not known whether this was a factor in the differences. We do not know the identity of the additional non-protein component of cells; however, it may be glycogen, lipid or salts. These findings illustrate the potential danger in using protein as the denominator when comparing various biochemical processes between species.

A number of subcellular processes were maximally inhibited and the proportion of hepatocyte respiration devoted to the activity was estimated by the reduction in the rate of hepatocyte oxygen consumption following such inhibition. All of these subcellular processes, total mitochondrial ATP production, ATP production to run the sodium pump, mitochondrial proton leak and non-mitochondrial oxygen consumption, showed negative allometric relationships with body mass (Fig. 5). The allometric exponents for these relationships are respectively –0.09, –0.11, –0.07 and –0.15, which are all reasonably similar to and within the 95% confidence limits of the exponent (–0.10 + 0.06) for total

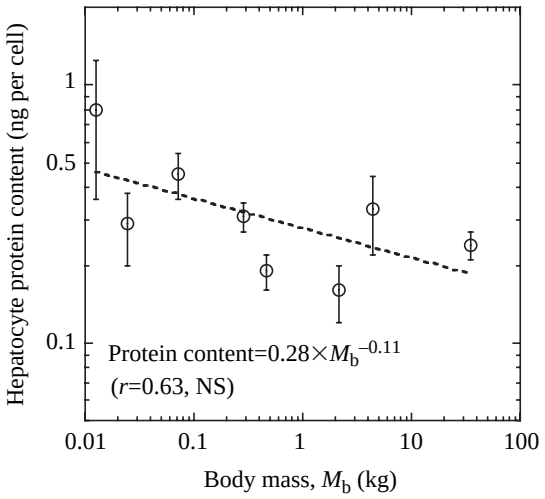

Fig. 4. The relationship between the calculated cellular protein content (ng) of hepatocytes and body mass (kg) of different-sized birds. Values are means ± S.E.M. N=4 for all bird species except finches and pigeons (N=3).

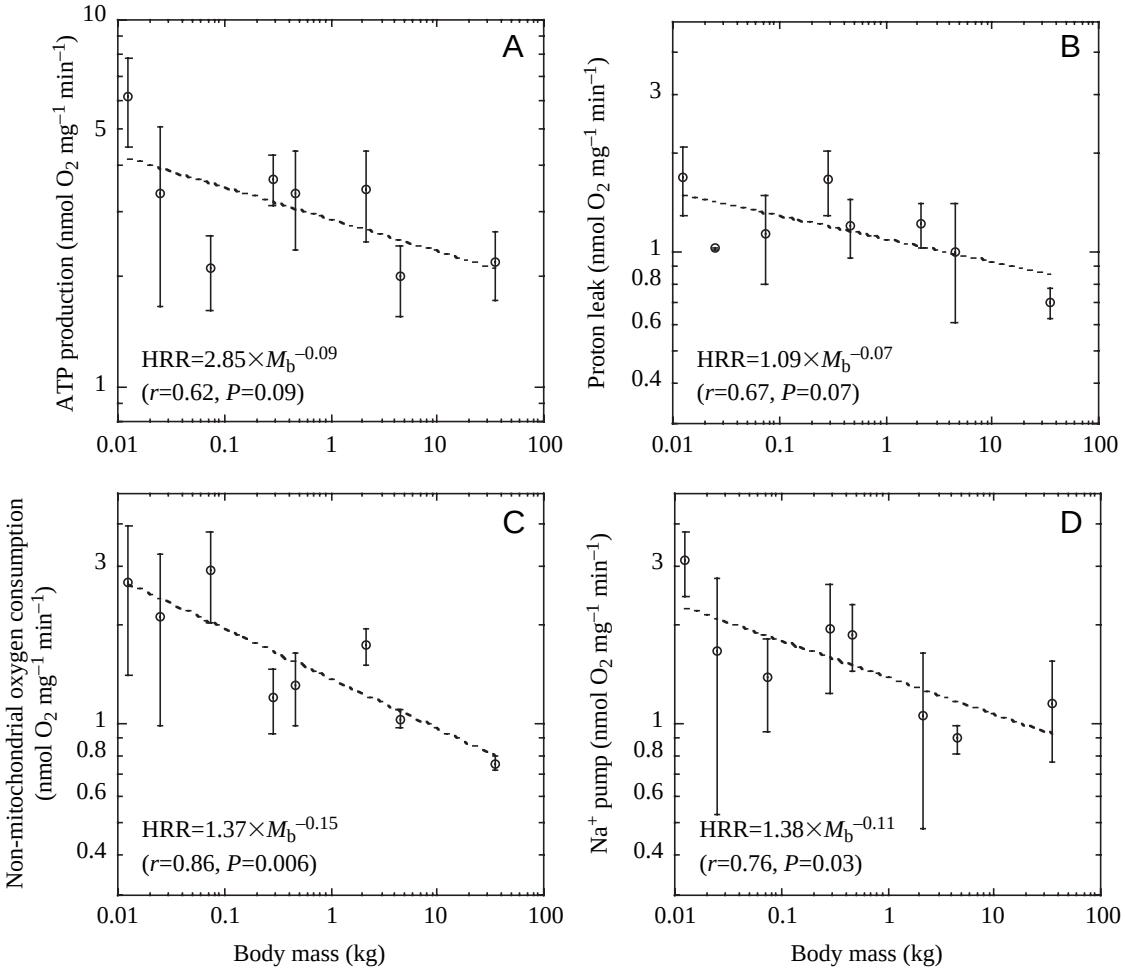

Fig. 5. The allometric relationship between the amount of the respiration rate of hepatocytes (HRR) devoted to four subcellular activities and body mass of different-sized birds. The subcellular activities are: mitochondrial ATP production (A), mitochondrial proton leak (B), non-mitochondrial oxygen consumption (C), and the activity of the Na^+ pump (D). Values are means ± S.E.M. N=4 for all bird species except for geese (N=3) and sparrows (N=2), and (in B only) starlings (N=3) and finches (N=2).

hepatocyte respiration. This shows that irrespective of body size of the bird species, these processes represent approximately the same proportion of total hepatocyte respiration. The exponents for ATP production and proton leak were not statistically different from zero using a 5% significance level, but would be if a 10% significance level was used. The constant in each of the allometric equations in Fig. 5 is the value of the process in a 1 kg bird. From these values, it can be calculated that, for a 1 kg bird, total mitochondrial ATP production constitutes 54%, mitochondrial proton leak is 21% and non-mitochondrial oxygen consumption is 25% of hepatocyte respiration. The non-mitochondrial value is a composite of oxygen consumption by other subcellular organelles such as peroxisomes and by non-mitochondrial enzyme systems such as the desaturases. The energy consumption by the Na^+ pump in liver cells in culture constitutes 45% of total hepatocyte ATP production and 24% of total hepatocyte respiration.

By combining hepatocyte respiration rate with the total mass of the liver in each bird (and assuming that liver is a constant 20% dry mass in all birds) we have been able to estimate the resting oxygen consumption by the total liver for each bird. Fig. 6 presents an allometric plot of this estimated total liver respiration against body mass for the different-sized bird species measured in the present study. The allometric slope of 0.84 for this relationship suggests that respiration by the liver represents a larger proportion of total respiration in bird species of greater body mass in general or it may reflect a difference between passerines and non-passerines. Using the relationship between BMR of the birds examined here, presented in Fig. 1, we can calculate that in a 13 g zebra finch the liver consumes about 2% of the total oxygen consumed by the bird, whilst in a 35 kg emu the corresponding value is 7%. These values are likely to be underestimates, however, because hepatocytes in culture are presumably only consuming energy for self-maintenance, whilst the whole liver in the bird will be consuming additional energy on behalf of other body cells *via* the maintenance of blood composition.

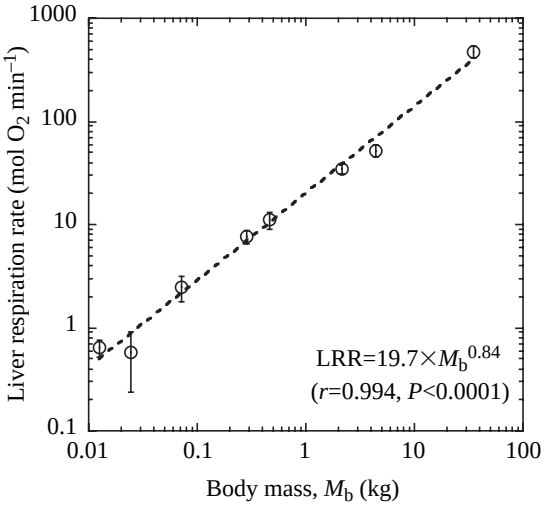

Fig. 6. The allometric relationship between calculated resting respiration rate of the whole liver (LRR) in different-sized birds and body mass. Values are means ± S.E.M. N=4 for all bird species except sparrows (N=2).

Discussion

This study has shown that, in agreement with the higher mass-specific basal metabolic rate of small species compared to larger species of birds, the respiration rate of isolated liver cells (per unit dry mass) from the smaller birds is greater than that of isolated liver cells from larger species. In this respect, it has demonstrated a similar trend to that already reported for mammals (Porter and Brand, 1995) and a disparate group of ectotherms (Hulbert et al., 2002a; although in the ectotherm allometric comparison the relationship was only significant at P=0.077). Similar allometric declines with increasing body mass have been reported in respiration rates of tissue slices for mammals (Couture and Hulbert, 1995), with mammalian liver slices showing a very similar allometric exponent to that for isolated mammal hepatocytes (Porter and Brand, 1995) and mammal kidney cortex slices (Couture and Hulbert, 1995) displaying almost the same exponent as that reported here for bird hepatocytes.

The hepatocyte respiration rate comparisons are illustrated in Fig. 7A, where it can be seen that the relationship for birds had a lower allometric slope than that for either mammals or ectotherms. Whether the size-related trend in viabilities of the hepatocyte preparations in this study (see Materials and methods) had an influence on the observed allometric relationship is not known. Another unknown factor is the fact that respiration rates in the present study were measured at the common temperature of 39–40°C. The smaller species in the present study were passerines, which tend to have higher body temperatures than the non-passerines representing the larger species we have measured here. Thus a Q_{10} correction of the current respiration data to the normal body temperature of the species will probably increase the rates for the small species and decrease those for the larger species with the consequent effect of increasing the allometric exponent relating hepatocyte respiration rate to body mass in birds.

The absolute and relative composition of hepatocyte respiration for a large and small species of both birds and mammals are plotted in Fig. 7Bi and ii, respectively. Whereas mass-specific respiration differs with body size in birds and mammals (Fig. 7A), when hepatocyte respiration components are compared in relative terms (Fig. 7Bii) the body size effect is no longer obvious and there is a very similar relative composition of metabolism between small and large birds and mammals, even though the non-mitochondrial component of

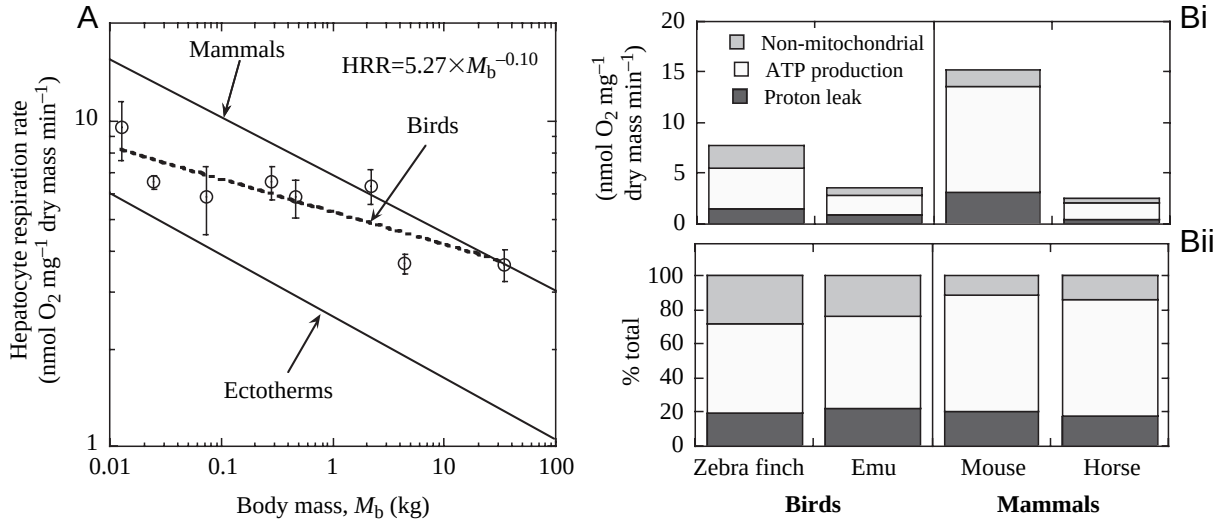

Fig. 7. A comparison of hepatocyte respiration in birds and mammals. (A) An allometric plot of the hepatocyte respiration rates (HRR) for different-sized birds (broken line) compared to the relationships (solid lines) previously described for mammals (from Porter and Brand, 1995) and ectotherms (from Hulbert et al., 2002a). (B) The composition of hepatocyte respiration for two birds (zebra finch and emu) and two mammals (mouse and horse). (Bi) The absolute values (in nmol O_2 mg^{-1} dry mass min^{-1}) for these four species; (Bii) the relative composition for the same species.

hepatocyte oxygen consumption may be greater in birds than mammals.

The amount of hepatocyte metabolism devoted to cellular Na^+ homeostasis in the different bird species was estimated by measurement of the reduction in oxygen consumption in the presence of excess ouabain (a specific inhibitor of the Na^+/K^+-ATPase). This rate also showed an allometric decline with increasing body mass (see Fig. 5). Since, when measured in this manner, this is not a measure of the total enzyme activity of Na^+/K^+-ATPase but is rather a measure of the *in vivo* activity of the sodium pump to maintain intracellular Na^+ homeostasis, it suggests that the relative 'sodium leak' is greater in hepatocytes from small birds than from large bird species. Measurement of sodium pump activity in liver and kidney slices of mammals of different body size has similarly shown an allometric decline with increasing body mass (Couture and Hulbert, 1995).

The findings of the present study parallel those previously reported for mammals in many respects. The mechanisms underlying these relationships are not precisely known but the 'membrane pacemaker' theory of metabolism (Hulbert and Else, 1999, 2000) proposes that both the amount and composition of cellular membranes underly such allometric variation in metabolic activity. Specifically, it proposes that polyunsaturated membranes are associated with high metabolic activity while more monounsaturated membranes are associated with lower metabolic activity. In this respect, birds show the same relationships as previously observed in mammals. We have previously reported that the muscle phospholipids of the same birds measured in the present study show these trends in fatty acid composition (Hulbert et al., 2002b) and have also found similar trends in the fatty acid composition of liver mitochondrial membranes from these birds (Brand et al., 2003). These trends in membrane fatty acid composition also exist in other tissues (A. J. Hulbert, N. Turner, M. D. Brand and P. L. Else, unpublished findings).

Recently, as described in the Introduction, there has been disagreement in the scientific literature between two schools of thought concerning the explanation of the metabolism–body size allometric relationship. One school has proposed a theory to explain this relationship based on the mathematics of fractal-like distribution networks (West et al., 1997, 2002) whilst the other school has suggested a multiple-causes model, where the final exponent describing the metabolism body mass exponent is the sum of multiple contributors to metabolism and control. This has been called the allometric cascade explanation (Darveau et al., 2002). The data from the present study undoubtably supports the 'allometric cascade' model. We have found a significant allometric relationship describing the mass-specific respiration rate of bird hepatocytes that is less than that describing the basal metabolic rate of whole birds. We have found that the total size of the liver also varies allometrically with body size in birds and that when liver mass and the hepatocyte respiration rate are combined they come closer to the BMR–body mass relationship. In this respect, our findings are similar to those already reported for mammals (Porter and Brand, 1995; Couture and Hulbert, 1995). There is sure to be more discussion regarding the causal basis of the allometric relationship between body size and metabolism.

We thank Dr P. Abolhasan and Dr W. A. Buttemer for assistance. This work was supported by a grant from the Australian Research Council (to A.J.H. and P.L.E.) and funds from the UK Medical Research Council (to M.D.B.).

References

Brand, M. D., Turner, N., Ocloo, A., Else, P. L. and Hulbert, A. J. (2003). Proton conductance and fatty acyl composition of liver mitochondria correlates with body mass in birds. *Biochem. J.* **376**, 741-748.

Brody, S. and Procter, R. C. (1932). Relation between basal metabolism and mature body weight in different species of mammals and birds. *Univ. Missouri Agric. Exp. Sta. Res. Bull.* 115pp.

Brody, S. (1945). *Bioenergetics and Growth.* Canada: Hafner Press. 1023pp.

Couture, P. C. and Hulbert, A. J. (1995). Relationship between body mass, tissue metabolic rate, and sodium pump activity in mammalian liver and kidney. *Am. J. Physiol.* **268**, R641-R650.

Darveau, C. A., Suarez, R. K., Andrews, R. D. and Hochachka, P. W. (2002). Allometric cascades as a unifying principle of body mass effects on metabolism. *Nature* **417**, 166-170.

Hulbert, A. J. and Else, P. L. (1999). Membranes as possible pacemakers of metabolism. *J. Theor. Biol.* **199**, 257-274.

Hulbert, A. J. and Else, P. L. (2000). Mechanisms underlying the cost of living in animals. *Ann. Rev. Physiol.* **62**, 207-235.

Hulbert, A. J., Else, P. L., Manolis, S. C. and Brand, M. D. (2002a). Proton leak in hepatocytes and liver mitochondria from archosaurs (crocodiles) and allometric relationships for ectotherms. *J. Comp. Physiol. B* **172**, 387-397.

Hulbert, A. J., Faulks, S. C., Buttemer, W. A. and Else, P. L. (2002b). Acyl composition of muscle membranes varies with body size in birds. *J. Exp. Biol.* **205**, 3561-3569.

Kleiber, M. (1932). Body size and metabolism. *Hilgardia* **6**, 315-353.

Lasiewski, R. C. and Dawson, W. R. (1967). A re-examination of the relation between standard metabolic rate and body weight in birds. *Condor* **69**, 13-23.

Maloney, S. and Dawson, T. J. (1993). Sexual dimorphism in BMR and T_b of a large bird, the Emu. *Condor* **95**, 1034-1037.

Porter, R. K. and Brand, M. D. (1995). Causes of differences in respiration rate of hepatocytes from mammals of different body mass. *Am. J. Physiol.* **269**, R1213-R1224.

West, G. B., Brown, J. H. and Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122-126.

West, G. B., Woodruff, W. H. and Brown, J. H. (2002). Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. *Proc. Natl. Acad. Sci. USA* **99** (supplement 1), 2473-2478.

Withers, P. (1992). *Comparative Animal Physiology*. Fort Worth, TX USA: Saunders Publishing.