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Review

Multi-level regulation and metabolic scaling

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Summary

Metabolic control analysis has revealed that flux through pathways is the consequence of system properties, i.e. shared control by multiple steps, as well as the kinetic effects of various pathways and processes over each other. This implies that the allometric scaling of flux rates must be understood in terms of properties that pertain to the regulation of flux rates. In contrast, proponents of models considering the scaling of branching or fractal-like systems suggest that supply rates determine metabolic rates. Therefore, the allometric scaling of supply alone provides a sufficient explanation for the allometric scaling of metabolism. Examination of empirical data from the literature of comparative physiology reveals that basal metabolic rates (BMR) are driven by rates of energy expenditure within internal organs and that the allometric scaling of BMR can be understood in terms of the scaling of the masses and metabolic rates of internal organs. Organ metabolic rates represent the sum of tissue metabolic rates while, within tissues, cellular metabolic rates are the outcome of shared regulation by multiple processes. Maximal metabolic rates (MMR, measured as maximum rates of O_2 consumption, $\dot{V}_{O_2max}$) during exercise also scale allometrically, are also subject to control by multiple processes, but are due mainly to O_2 consumption by locomotory muscles. Thus, analyses of the scaling of MMR must consider the scaling of both muscle mass and muscle energy expenditure. Consistent with the principle of symmorphosis, allometry in capacities for supply (the outcome of physical design constraints) is observed to be roughly matched by allometry in capacities for demand (i.e. for energy expenditure). However, physiological rates most often fall far below maximum capacities and are subject to multi-step regulation. Thus, mechanistic explanations for the scaling of BMR and MMR must consider the manner in which capacities are matched and how rates are regulated at multiple levels of biological organization.

Key words: metabolic regulation, respiration, mitochondria, BMR, $\dot{V}_{O_2max}$, allometry.

Introduction

Metabolic rates scale allometrically, such that a unit mass of elephant has a metabolic rate much less than that of a unit mass of mouse. To empiricists, the question 'what *causes* the allometric scaling of metabolic rates?' is one that is inextricably linked to the question 'what *determines* metabolic rates?' This is because whatever phenomena determine metabolic rates should presumably play major roles in driving their allometric scaling. The fundamental importance of both questions has been recognized by biologists for a century, and studies addressing them have had long and illustrious histories. Introductions to the phenomenon of metabolic scaling and accounts of the historical development of ideas and studies devoted to the subject can be found in excellent books (Calder, 1984; Schmidt-Nielsen, 1984) and reviews (Calder, 1981; Hoppeler et al., 1980; Porter, 2001; Taylor, 1987; Weibel, 1987) and are beyond the scope of this article. Here, we discuss some empirical data from the literature of comparative physiology to address the issue of what determines metabolic rates in animals. We relate this information to the allometric scaling of metabolic rates and comment on recently proposed models for metabolic scaling.

Animals as the sum of their parts

When data for basal metabolic rate, BMR, are plotted against body mass, M_b, on logarithmic coordinates, the slope of the linear relationship, referred to as the allometric exponent, b, in the equation:

$$\mathrm{BMR} = a \times M_b^{\,b}\,, \qquad (1)$$

is significantly less than 1.0, where a is the vertical intercept (Schmidt-Nielsen, 1984). Whether b is closer to 3/4 (Savage et al., 2004) or to 2/3 (Dodds et al., 2001; White and Seymour, 2003) is still hotly debated. The issue continues to be worthy

of debate, at least partly because of the mechanistic implications of what the allometric exponents happen to be (Suarez et al., 2004). Schmidt-Nielsen (1984) pointed out that regression lines and allometric equations are simply quantitative descriptions of data. It is, therefore, reasonable to expect that explanations for the scaling of metabolic rates, i.e. why the points lie where they do and why the slopes are as they are, should be consistent with what is known about how metabolic rates are regulated in animals.

Attempts to account for the allometry in BMR scaling by considering the sum of organ metabolic rates go at least as far back as the work of Krebs (1950). The rather simple idea behind this is that by considering the scaling of organ masses as well as organ metabolic rates, one should be able to explain the scaling of whole-body BMR. While the work of Krebs was limited by the use of *in vitro* metabolic rates, more recently Wang et al. (2001) used more physiologically relevant data obtained *in vivo*. Among mammals, the masses and metabolic rates of internal organs (liver, brain, kidneys and heart) account for a large fraction of BMR. Wang et al. (2001) found that with increasing body mass, this fraction declines, from 68% of BMR in a mammal weighing 100 g to 34% in one weighing 1000 kg. In their analysis, the rest of BMR is accounted for by 'remaining tissues', a category that includes skeletal muscles. The internal organs show variable mass-scaling exponents (from 0.76 for brains to 0.98 for hearts) and metabolic rate scaling exponents (–0.08 for kidneys to –0.27 for livers; Table 1). When the metabolic rates of these organs and the remaining tissues are summed, the outcome is a value for b of 0.76, which is remarkably close to the much-quoted exponent obtained by Kleiber (1932). The actual value of b, of course, is a subject of dispute and, given the relatively narrow body mass range considered by Wang et al. (2001), their findings serve mainly to illustrate that the allometric scaling of BMR can be accounted for by the scaling of organ masses and organ metabolic rates.

Table 1. *Allometric equations for the scaling of organ masses and metabolic rates in mammals*

Organ	Organ mass	SMR	Organ mass×SMR
Liver	$0.033M_b^{0.87}$	$2861M_b^{-0.27}$	$94.4M_b^{0.60}$
Brain	$0.011M_b^{0.76}$	$1868M_b^{-0.14}$	$20.5M_b^{0.62}$
Kidneys	$0.007M_b^{0.85}$	$2887M_b^{-0.08}$	$20.2M_b^{0.77}$
Heart	$0.006M_b^{0.98}$	$3725M_b^{-0.12}$	$22.4M_b^{0.86}$
Remainder	$0.939M_b^{1.01}$	$125M_b^{-0.17}$	$117.4M_b^{0.84}$

Data taken from Wang et al. (2001).

M_b, body mass in kg; SMR, specific metabolic rates in kJ kg^{-1} day^{-1}.

These relationships raise the question of why organs masses and specific metabolic rates decline with increasing body mass.

Does supply determine organ metabolic rates?

Given the allometric scaling of the metabolic rates of internal organs and their contributions to the scaling of BMR, it is relevant to consider what is currently known concerning what determines organ metabolic rates. Fig. 1 shows how mass-specific resting metabolic rates decline with increasing body mass in several species of small mammals (from Singer et al., 1995). Recently, it has been suggested that metabolic rates are determined by the rates of resource supply to cells *via* branching (Banavar et al., 2002) or fractal-like (West et al., 1997, 1999, 2002) structures. It is well-known to physiologists that blood flow is acutely adjusted to physiological needs such that internal organs in resting animals typically do not experience a limiting supply of O_2 or metabolic substrates. In addition, whole-body metabolic rates among mammals increase, on average, by about tenfold as they go from BMR to MMR (maximum aerobic metabolic rates, expressed as the maximum rate of O_2 consumption, $\dot{V}_{O_2max}$) during exercise (Weibel, 2000). More athletic species display even higher metabolic scopes (Jones and Lindstedt, 1993; Weibel, 2000). The presence of large excess capacities in the cardio-respiratory systems of mammals, by itself, should cast doubt upon the idea that the supply of materials through the circulation should limit rates of organ metabolism at rest. The data presented in Fig. 1 were obtained from hibernators. In the same species, the transition from the euthermic to hibernating state results in dramatic declines in (and, resulting isometry of) whole-body metabolic rates. This is not simply a passive effect of cooling, but the result of active downregulation of rates of energy expenditure within internal organs (Heldmaier and Elvert, 2004; Heldmaier and Ruf, 1992). It can be inferred from all these, as well as the lack of supply limitations, that the energy expenditure in internal organs determines their metabolic rates and contributions to whole-body BMR.

Cellular energy metabolism: lessons from control analysis

Organs consist of multiple tissues that, in turn, consist of specific cell types. The control of metabolism in hepatocytes, neurons, tubule cells, and cardiomyocytes therefore becomes a central issue to consider when determining what controls the rate of metabolism in livers, brains, kidneys, hearts and, ultimately, whole animals.

Early in the development of metabolic biochemistry as a discipline, break-through discoveries of individual enzyme-catalyzed reactions and the pathways they constitute were followed by measurements of flux rates and studies of their regulation. Monod's concept of *allosterie*, of such great importance that he referred to it as the 'second secret of life' (see Perutz, 1990), led to much research effort to identify rate-limiting steps and to elucidate how the activities of allosteric enzymes are altered in response to changes in the concentrations of modulators. After many years of such research, the concept of the rate-limiting step became problematic. For example, in the case of glycolysis, perhaps the best-studied of all pathways, multiple steps were found to be displaced from equilibrium and potentially rate-limiting, and several enzymes were discovered to be subject to various

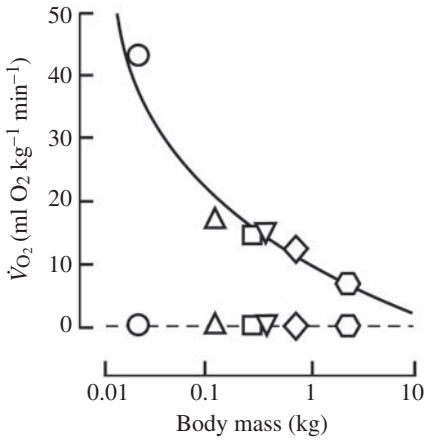

Fig. 1. Metabolic rate ($\dot{V}_{O_2}$) as a function of body mass in a number of small mammals (circle, hazel mouse; triangle, edible dormouse; square, ground squirrel; inverted triangle, European hamster; diamond, European hedgehog; hexagon, alpine marmot) during euthermy (solid line) or hibernation (broken line). Metabolic rate (BMR) scales allometrically during euthermia, but during hibernation BMR scales isometrically. The differences between euthermic and hibernating metabolic rates are due to alterations in the rates of energy expenditure in internal organs. Redrawn from Singer et al. (1995).

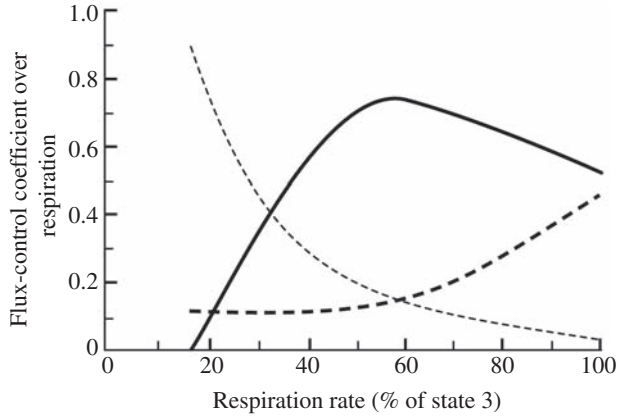

Fig. 2. Flux control coefficients of ATP turnover (solid line), substrate oxidation (thick broken line), and proton leak (thin broken line), showing shared and changing contributions to control as the system moves towards 100% of state 3 respiration rate. Results were obtained using top-down control analysis. Redrawn from Brand et al. (1993).

forms of regulation. Is there really just one rate-limiting step? Over the past three decades, much progress has been made towards a more sophisticated, quantitative understanding of the control of metabolism. A major factor contributing to this was the development of metabolic control theory and the application of metabolic control analysis to studies of the control of flux (Fell, 1992, 1997). Metabolic control theory considers the flux through a pathway as a system property that is subject to shared control by multiple steps. The degree of control at each step can be quantified by estimation of its flux control coefficient, C_i, which represents the degree to which a step, i, contributes to the regulation of flux, J, through a pathway. C_i for any step is expressed in terms of the fractional change in pathway flux ($\delta J/J$) that occurs in response to an infinitesimal fractional change in the rate of enzyme activity ($\delta e_i/e_i$):

$$C_i = (\delta J/J) / (\delta e_i/e_i) . \quad (2)$$

There are now many examples of the application of metabolic control analysis to studies of the control of flux through various pathways (Fell, 1997). The prediction, based on theoretical considerations, that control should be shared by multiple steps, is now supported by a large body of empirical evidence. For example, arguments concerning what is rate-limiting in glycolysis are now informed by quantitative data. Using a bottom-up approach that involves estimating C_i values from the elasticities (i.e. enzyme kinetic responses to variation in substrate concentration) at each of the steps, Kashiwaya et al. (1994) found not only distribution of control among multiple steps, but also changes in C_i values as hearts were perfused with or without insulin and with single or multiple substrates. Thus, the control of flux is shared by multiple steps whose relative contributions can change in response to changes in physiological conditions.

Under steady-state, aerobic conditions, most of the total cellular O_2 consumption is due to mitochondrial respiration (for the present discussion, we shall ignore the small fraction due to non-mitochondrial processes). Because energy metabolism is inherently complex, addressing the question of what controls mitochondrial O_2 consumption in intact cells is potentially more difficult than determining C_i values for individual steps in linear pathways. To a large extent, the difficulties have been surmounted by the use of top-down metabolic control analysis. This is a simplifying approach, developed by Brand and colleagues (Brand, 1996), which involves conceptually subdividing metabolism into blocks consisting of entire pathways, networks of pathways or groups of reactions. Individual blocks are considered to be linked to each other *via* a common intermediate, and the strength of control of blocks over each other is estimated empirically. In isolated liver mitochondria, Hafner et al. (1990) designated the group of reactions involving substrate oxidation that create the proton-motive force, Δp, as one such block. Two other blocks were designated as those involved in dissipating Δp, i.e. proton leak and the phosphorylation system (i.e. ATP synthesis). The kinetic responses of these blocks, measured as O_2 consumption, to changes in their common intermediate, Δp, were determined. Fig. 2 plots the C_i values of substrate oxidation, proton leak and phosphorylation systems on mitochondrial respiration as a function of percent of the state 3 rate (maximum rate of O_2 consumption coupled to ATP synthesis). It is seen that non-phosphorylating mitochondria (state 4) respire at between 10–20% of the maximum rate. Under these conditions, substrate oxidation accounts for a small fraction and proton leak accounts for most of the control of respiration, while ATP synthesis has no influence. As mitochondria approach 100% of their state 3 respiration rate,

control by proton leak declines while control by ATP synthesis increases. Control of substrate oxidation increases more gradually until it shares, along with ATP synthesis, most of control, while proton leak has minimal influence over respiration at 100% of state 3.

In contrast with isolated mitochondria, both ATP synthesis and hydrolysis occur simultaneously and might be expected to regulate each other in intact cells. Applying the top-down approach to the control of respiration in isolated rat hepatocytes, Brown et al. (1990) estimated C_i values of 0.29 for the processes that generate Δp, 0.49 for the processes that synthesize, transport and use ATP, and 0.22 for the proton leak. In isolated perfused rat livers in the 'resting' state, i.e. when no substrates for gluconeogenesis or ureagenesis are provided, Soboll et al. (1998) found that mitochondrial respiration is controlled by 'maintenance' ATP-hydrolyzing reactions, while mitochondrial reactions involved in ATP synthesis have no influence. However, when livers are made to synthesize glucose and urea at high rates, mitochondrial ATP synthesis exerts strong control over its own rate as well as on both gluconeogenesis and ureagenesis. In addition, both gluconeogenesis and ureagenesis exert negative control over each other's rates, presumably by competing for ATP. In such active livers, maintenance ATP-hydrolyzing reactions are unaffected by rates of mitochondrial ATP supply and demand by other processes, but exert control over all other pathways. It is likely that *in vivo*, when livers are actually performing their physiological functions, control of mitochondrial O_2 consumption is shared by ATP-requiring biosynthetic and maintenance reactions with processes involved in ATP synthesis and proton leak (Rolfe et al., 1999).

Mammalian kidneys are estimated to have 'basal' rates of O_2 consumption that are between 3–18% of the normal physiological rates expressed when active ion transport is occurring. Experiments involving manipulation of rates of Na^+ transport yield a positive, linear relationship between transport and O_2 consumption rates, and such results have been used to calculate the ATP cost of Na^+ pumping by Na^+-K^+-ATPase (Mandel and Balaban, 1981). Although control analysis has not been conducted on perfused kidneys or kidney tubules *in vitro*, it is apparent that the metabolic rates of kidneys are controlled by rates of energy expenditure, i.e. ATP hydrolysis, driven primarily by active ion transport.

Arrested hearts consume O_2 at only 15% of the rates seen in normal, working hearts. Therefore, about 85% of cardiac metabolic rate represents the energetic cost of performing mechanical work plus the cost of excitation–contraction coupling (Rolfe and Brown, 1997). Cardiac $\dot{V}_{O_2}$ increases linearly with work rate, and intracellular free Ca^{2+} may be involved in the concerted regulation of both $\dot{V}_{O_2}$ as well as work rate (Balaban and Heineman, 1989; Territo et al., 2001). In recent work involving top-down control analysis of cardiac energy metabolism, Diolez et al. (2000, 2002) designated ATP-synthesizing and hydrolyzing reactions as two separate blocks, linked by their common intermediate, ATP. Using perfused rat hearts, they found that ATP hydrolysis accounts for about 90% of the control of respiration, while the remainder is accounted for by ATP synthesis. Energy metabolism in cardiac tissue is not limited by the supply of O_2 or substrates (Mootha et al., 1997; Zhang et al., 1999). These findings, as well as the results of Diolez et al. (2002), provide quantitative support for the widely held view that energy expenditure (i.e. work rate), rather than the rate of material supply, sets the pace for cardiac energy metabolism.

The control of $\dot{V}_{O_2max}$

The relative contributions of various organs to whole body $\dot{V}_{O_2}$ change dramatically as animals go from their basal to maximal, aerobic metabolic rates, expressed as $\dot{V}_{O_2max}$ (Weibel, 2002). Here, we consider $\dot{V}_{O_2max}$ achieved during exercise (although some animals are known to achieve $\dot{V}_{O_2max}$ in other circumstances, e.g. pythons during digestion of food; Secor and Diamond, 1995). Under these conditions, cardiac output increases to values several-fold higher than at rest and most of the increase in blood flow is directed to locomotory muscles. At $\dot{V}_{O_2max}$ during exercise, skeletal muscle mitochondria are responsible for 90% or more of whole body O_2 consumption rate (Taylor, 1987).

Control analysis, performed using isolated muscle mitochondria, yields results similar to those shown in Fig. 2 (Brand et al., 1993). A more 'complete' system, consisting of skinned muscle fibers, has also been used wherein actomyosin-ATPase activities and mitochondrial oxidative phosphorylation can be varied by manipulation of free Ca^{2+} concentration. Control analysis, applied to such preparations, reveals that about half of the control of respiration resides in actomyosin-ATPase, while the balance is accounted for by the mitochondrial adenine nucleotide translocase and mitochondrial reactions involved in electron transport (Wisniewski et al., 1995).

Control analysis has been applied by Brown (1994) to the control by organs of the concentrations and flux rates of metabolites in the blood. This approach is of heuristic value and was applied by Brown (1994) to analyze ketone body metabolism. However, simplifying assumptions made concerning the route of blood flow and the (quite understandable) lack of incorporation of cardio-respiratory parameters precludes the application of this particular approach to the analysis of the control of $\dot{V}_{O_2max}$. Nevertheless, over the years, a number of forms of control analysis have been adopted in studies of the respiratory physiology of exercise. These studies reveal that, during exercise at $\dot{V}_{O_2max}$, the rate of transport of materials by branching or fractal-like structures does play a role in limiting whole body metabolic rate. Using an approach that is most closely analogous to the control analysis performed by metabolic biochemists, Jones (1998) estimated control coefficients for $\dot{V}_{O_2max}$ in thoroughbred racehorses of 0.309, 0.308, 0.263 and 0.120 for ventilation rate, pulmonary O_2 diffusing capacity, cardiac output, and muscle O_2 diffusing capacity, respectively. Although differing in methodological details and in the species used, these findings

are consistent with those obtained by others (e.g. di Prampero, 1985; Wagner, 1993, 1996); i.e. control analysis of whole animal $\dot{V}_{O_2max}$ reveals that control is shared among the convective and diffusive steps in the transport of O_2 from the external environment, through the lungs and circulation, to the muscle mitochondria.

Causation in metabolic scaling

In recent years, the publication and popularization of proposed explanations (Banavar et al., 2002; West et al., 1997, 1999, 2002) for metabolic scaling have stimulated renewed interest in the subject. The model of West et al. (1997), in particular, serves as the basis for what is now referred to as a mechanistic 'metabolic theory of ecology'. Metabolic rate as mass to the 3/4 power (and a correction factor for temperature) might be good enough for the purposes of ecosystem ecologists, regardless of what the underlying mechanism(s) that drive the relationships might be. However, are such models truly mechanistic in the sense that they reflect the causal relationships that bring about metabolic rates and their scaling?

An inherent problem with the models proposed by both Banavar et al. (2002) and West et al. (1997) is that both are based on the assumption that metabolic rates are supply-limited. Thus, according to their logic, a model for the scaling of supply rates serves as the explanation for the scaling of metabolic rates. West et al. (2002) state this explicitly: '*A quantitative theoretical model (West et al., 1997) has been developed that accounts for quarter-power scaling on the basis of the assumption that metabolic rates are constrained by the rate of resource supply*.' Similar reasoning led Banavar et al. (2002) to argue that '*intracellular processes and properties – including the rates of chemical reactions in organelles, the function and concentration of enzymes, and the strength of chemical bonds – are most unlikely to exhibit necessary changes in direct response to the overall size of the organism.*' They add that '*The correspondence between the scaling exponent for the capacity of the circulatory system and that observed for overall metabolism*' leads to the conclusion that '*the rates of intracellular metabolic-related processes conform roughly to the scaling of the supply network and exert little, if any, net effect on the scaling of overall metabolism*.'

If supply rates do not regulate BMR, then models describing the scaling of supply rates alone cannot provide adequate explanations for the scaling of BMR (Suarez et al., 2004). In their paper describing the scaling of BMR on the basis of the organ mass and metabolic rate scaling, Wang et al. (2001) appropriately pointed out the importance of determining the mechanisms that drive the allometric scaling of organ metabolic rates.

It turns out that, contrary to the assertions of proponents of supply-based models of metabolic scaling, the metabolic rates of cells isolated from mammals (Porter, 2001), birds (Else et al., 2004), reptiles and archosaurs (Hulbert et al., 2002) decline with increasing body mass. There is, in fact, much work directed towards investigation of the biochemical bases for the allometric scaling of cellular metabolism (reviewed by Suarez et al., 2004). Because rates of cellular energy expenditure scale allometrically, we developed a 'multiple-cause' explanation as an alternative to 'single-cause' supply-based explanations for metabolic scaling (Darveau et al., 2002; Hochachka et al., 2003). In essence, the idea is that metabolic scaling is the consequence of the contributions of various processes involved in both the supply of materials and energy expenditure to the control of $\dot{V}_{O_2}$. Although formal presentation of this concept (called the 'allometric cascade') is beyond the scope of the present paper, in light of the preceding sections, it should be apparent, if supply does not limit BMR, that multiple contributors to, and the relative strengths of their control over, whole body metabolic rates must be considered to account for BMR. The allometric cascade has been challenged (Banavar et al., 2003; West et al., 2003) on the basis of mathematical flaws; these and other limitations have been acknowledged by us (Darveau et al., 2003). Nevertheless, the physiological and biochemical bases are valid, and the concept itself remains unchallenged.

In principle, the same arguments should apply to the allometric scaling of $\dot{V}_{O_2max}$, a condition wherein the supply of materials *via* branching or fractal-like networks plays an increased role in limiting whole-body metabolic rate. It is interesting that despite this, $\dot{V}_{O_2max}$ in mammals scales with an exponent of about 0.86 (Taylor et al., 1989; Weibel et al., 2004), a value significantly higher than the exponent of 0.75 predicted by models that assume supply limitation. Mitochondrial respiration rates in exercising muscles are mass-independent, at about 5 ml O_2 cm^{-3} mitochondria min^{-1} (Hoppeler and Lindstedt, 1985; Taylor et al., 1989; Weibel et al., 2004). The isometry of mitochondrial respiration rates *in vivo* at $\dot{V}_{O_2max}$ has been interpreted to mean that the delivery of O_2 is adjusted *via* adaptations in structure and function such that, on average, muscle mitochondria in large species are as well supplied with O_2 as those in small species. Large animals, therefore, are not disadvantaged relative to small animals with respect to their abilities to supply the requirements of their mitochondria under basal or maximal aerobic rates of metabolism. Rather, supply matches demand, and demand is set by the rate of energy expenditure. We are led to conclude that the scaling of muscle mass and the processes involved in muscle energy expenditure must be considered in addition to the scaling of supply rates to explain the allometric scaling of $\dot{V}_{O_2max}$ (Suarez et al., 2004). Such a shift from considering only the scaling of supply rates to consideration of the scaling of energy expenditure provides an explanation for deviations from 3/4 power scaling exponents commonly observed in intra- and interspecific studies. Much of the deviation is the outcome of evolutionary adaptation to lifestyles and environments (Childress and Somero, 1990; Suarez et al., 2004).

Capacities *vs* rates

In the present context, flux rates are viewed as being acutely regulated to satisfy physiological requirements. Capacities, on

the other hand, represent the upper limits to flux rates (Suarez et al., 1997). Although rates and capacities are different, focus on the allometric scaling of rates has resulted in relative neglect of the scaling of capacities and the significance of this. At the level of each enzyme-catalyzed step in pathways, the maximum capacity for flux, V_{max}, is a function of enzyme concentration, [*E*], and catalytic efficiency, k_{cat}, such that $V_{max}=[E]\times k_{cat}$. Regardless of the degree of displacement from equilibrium of the reactions they catalyze, most metabolic enzymes operate far below their maximal capacities *in vivo* (Suarez et al., 1997). Their operation at low fractional saturation of substrate binding sites makes possible regulation by substrate (and product) concentrations, as well as by alterations in binding affinities *via* allosteric mechanisms or covalent modification. This also enables metabolic enzymes to regulate, within very narrow ranges, the concentrations of metabolic intermediates in pathways (Atkinson, 1977; Fell, 1997).

Species with similar body temperatures possess enzyme orthologues with similar k_{cat} values (Hochachka and Somero, 2002), so among such animals, the intra- and interspecific variation in V_{max} values at any particular step in metabolism is mainly the result of variation in [*E*]. Allometric variation in flux capacities is widespread. In various organs, capacities for membrane ion pumping, i.e. V_{max} values for Na^+-K^+-ATPase (Couture and Hulbert, 1995) and Ca^{2+}-ATPase (Hamilton and Ianuzzo, 1991) decline with increasing mass. Allometry in V_{max} values for citrate synthase, a Krebs cycle enzyme that serves as an index of oxidative capacity, is observed in the skeletal muscles of mammals (Emmett and Hochachka, 1981) and fishes (Childress and Somero, 1990; Somero and Childress, 1990). Consistent with these patterns is the decline in mitochondrial content observed in skeletal muscle, heart, liver, kidney and brain with increasing mass (Else and Hulbert, 1985a,b; Hoppeler et al., 1984; Mathieu et al., 1981). Because enzymes and mitochondria usually do not operate at their maximum capacities, allometry in flux capacities provides only a partial explanation for allometry in cellular O_2 consumption rates. Further insights can be derived from the work of Porter (2001) and Else et al. (2004), who found decreasing rates of proton leak and ATP turnover (i.e. hydrolysis by ATPases matched by rates of mitochondrial oxidative phosphorylation) in mammalian and avian hepatocytes as a function of increasing body mass. Thus, biochemical *capacities* (the outcome of both ontogeny and phylogeny) as well as the actual *rates* of ATP hydrolysis and oxidative phosphorylation (‘system properties’, controlled by multiple steps) decline with increasing body mass.

Why do the rates decline? Pioneering studies of the regulatory mechanisms that drive these allometric relationships in mammalian hearts have been conducted by Dobson and colleagues (Dobson, 2003; Dobson and Headrick, 1995; Dobson and Himmelreich, 2002). Cardiac work rates and, therefore, rates of ATP hydrolysis per unit mass of cardiac tissue, decline with increasing body mass. The allometric scaling of cardiac energy expenditure is reflected in the scaling of bioenergetic parameters that are thought to be involved in regulating mitochondrial ATP synthesis. Cytosolic free ADP concentrations in cardiac ventricles increase with body mass, such that 1/[ADP] scales as $M_b^{-0.23}$ and the cytosolic phosphorylation potential, [ATP]/[ADP][P_i], scales as $M_b^{-0.28}$. Dobson et al. suggest that, in smaller animals, the higher phosphorylation potential results in a higher Gibbs free energy of ATP hydrolysis as well as a higher ‘kinetic gain’, wherein small fractional changes in [ADP] result in greater fractional changes in cardiac V_{O_2} relative to larger animals.

It is reasonable to expect, given the diversity of animals and their adaptations to lifestyles and environments, that there should be many reasons for the allometric scaling of rates and capacities at multiple levels of organization (Suarez et al., 2004). Although physical laws may dictate that capacities for the supply of materials to cells *via* branching or fractal-like structures must decline with increasing body mass, the lack of control of whole body BMR by the supply of materials, as well as its only partial control over V_{O_2max}, require a re-examination of the consequences of allometry in capacities for delivery.

Metabolic scaling and symmorphosis

More than two decades ago, Taylor and Weibel (1981) proposed symmorphosis, a concept that says animals are designed economically such that structures and functional capacities satisfy, but do not exceed, maximum physiological requirements or ‘loads’. The proposal that capacities should match maximum loads is, at least implicitly, an optimality hypothesis that says natural selection eliminates excess capacities. It also predicts that capacities in multi-step processes or pathways should be matched to each other. It is important to digress by pointing out that symmorphosis has provoked considerable controversy, mainly because of its evolutionary implications. Favourable views include (and here is a caricature), ‘*Symmorphosis is so obviously correct that there is no need for the term and no need to study the phenomenon; it is like saying that animals that need big feet have big feet*’. Another, consistent with the use of optimality theory in evolutionary biology (Parker and Maynard Smith, 1990), is that symmorphosis is a good starting hypothesis and deviation from its predictions is informative (Diamond, 1992). On the other hand, critics state that the concept is naïve because optimal matches between capacities and maximum physiological requirements are, in principle, unlikely to result from natural selection and are, in reality, not observed (Dudley and Gans, 1991; Garland and Huey, 1987). This is the position often taken by evolutionary physiologists, some of whom suggest that ‘adequacy’ or ‘sufficiency’ (Gans, 1993) is a better way of looking at animal design than optimality. Attempts to rigorously test symmorphosis as a hypothesis have yielded a broad spectrum of outcomes, and Garland (1998) has presented ideas concerning how proper interspecific tests of symmorphosis should be conducted. In the cardio-respiratory system, Weibel et al. (1991) report that there is excess capacity for O_2 flux in mammalian lungs, unlike other steps where there

are close matches between physiological requirements at $\dot{V}_{O2max}$ and capacities for O_2 transport. This interpretation has been challenged by Garland and Huey (1987). In a survey of available information from many different physiological systems, Diamond (1998) found excess capacities, i.e. 'safety factors', virtually everywhere in the vertebrate body and at all levels of biological organization.

Debates concerning symmorphosis have subsided and the concept has come to be regarded by some as a useful design principle, rather than a hypothesis with precise and rigid criteria for acceptance or rejection. In this spirit, the data available to comparative physiologists and biochemists (summarized in Suarez et al., 2004) can be seen as consistent with the idea that allometry, in capacities for the supply of oxidative fuels and O_2 to cells by branching or fractal-like networks, has been matched, through evolution, by allometry in capacities for substrate oxidation, aerobic ATP synthesis and ATP utilization. There are many mechanistic reasons for why elephants should not consist of cells with the same biochemical capacities as the cells in mice, and *vice versa*. However, just as homeostatic mechanisms have evolved to ensure that rates of ATP hydrolysis are matched by the rates of ATP synthesis in cells, at higher levels of organization, regulatory mechanisms have also evolved to ensure that delivery and utilization rates are matched to each other at rest as well as during steady state, aerobic exercise. It is, perhaps, for this reason (among others) that natural selection has not produced exact matches among functional capacities at various steps in oxygen transport.

Supply and demand systems are better viewed as having coevolved with each other, as having developed as interacting systems during ontogeny, and as exerting acute regulatory influences upon each other in living animals. Metabolic scaling is such a wonderful, many-splendoured thing that models based on supply limitation alone fail to do it justice. Progress towards a deeper understanding of the multiple causes of the allometric scaling of metabolism depends upon further advances in our understanding of the nature of structural and functional integration in animals, i.e. our ability to put Humpty Dumpty together again (Schultz, 1996).

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