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LIMITS FOR OXYGEN AND SUBSTRATE TRANSPORT IN MAMMALS

H. HOPPELER* AND E. R. WEIBEL
Institute of Anatomy, Bühlstrasse 26, CH-3000 Bern 9, Switzerland
*e-mail: hoppeler@ana.unibe.ch

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Summary

Environmental oxygen is transported by the respiratory cascade to the site of oxidation in active tissues. Under conditions of heavy exercise, it is ultimately the working skeletal muscle cells that set the aerobic demand because over 90 % of energy is spent in muscle cells. The pathways for oxygen and substrates converge in muscle mitochondria. In mammals, a structural limitation of carbohydrate and lipid transfer from the microvascular system to the muscle cells is reached at a moderate work intensity (i.e. at 40–50 % of $\dot{V}_{O_2max}$). At higher work rates, intracellular substrate stores must be used for oxidation. Because of the importance of these intracellular stores for aerobic work, we find larger intramyocellular substrate stores in 'athletic' species as well as in endurance-trained human athletes. The transfer limitations for carbohydrates and lipids at the level of the sarcolemma imply that the design of the respiratory cascade from lungs to muscle mitochondria reflects primarily oxygen demand. Comparative studies indicate that the oxidative capacity of skeletal muscle tissue, and hence maximal oxygen demand, is adjusted by varying mitochondrial content. At the level of microcirculatory oxygen supply, it is found that muscle tissue capillarity is adjusted to muscle oxygen demand but that the capillary erythrocyte volume also plays a role. Oxygen delivery by the heart has long been recognized to be a key link in the oxygen transport chain. In allometric variation it is heart rate and in adaptive variation it is essentially stroke volume, and hence heart size, that determines maximal cardiac output. Again, haematocrit is an important variable that allows the heart of athletic species to generate higher flux rates for oxygen. The pulmonary gas exchanger offers only a negligible resistance to oxygen flux to the periphery. However, in contrast to all other steps in the respiratory cascade, the lungs have only a minimal phenotypical plasticity and appear, therefore, to be built with considerable structural redundancy in all but the most athletic species. Because of the lack of malleability, the lungs may ultimately become limiting for $\dot{V}_{O_2max}$ when adaptive processes have maximized O_2 flux through the malleable downstream elements of the respiratory system: the heart, microcirculation and muscle mitochondria.

Key words: oxygen supply, substrate transport, mammal, exercise, endurance, mitochondrion, scaling, allometry, morphometry, capillary, heart rate.

Introduction

The idea of a *single step* of the oxygen cascade limiting oxygen flow through the respiratory system has been a very attractive concept and has dominated physiological thinking until recently. Implicitly or explicitly, most physiologists assumed the heart to be the most important step in aerobic energy transfer. In the 1970s, data on the remarkable variability of skeletal muscle oxidative capacity and capillary supply with endurance exercise training became available (Hoppeler *et al.* 1973; Holloszy and Booth, 1976), which brought the importance of the periphery for $\dot{V}_{O_2max}$ into focus. The year-long debate on the relative importance of central *versus* peripheral limitations of $\dot{V}_{O_2max}$ was finally resolved in a paper entitled 'Metabolic and circulatory limitation to $\dot{V}_{O_2max}$ at the whole animal level' by diPrampero (1985). Using a deceptively simple algebraic model, he calculated the contribution of the individual steps of the respiratory cascade viewed as resistors in series. He could demonstrate that, for humans exercising in normoxia, approximately 75 % of $\dot{V}_{O_2max}$ is set by central O_2 transport and the remaining 25 % by the periphery. Additionally, he pointed out that the relative importance of the individual transfer steps was greatly affected by external conditions such as hypoxia, work with small muscle groups, etc. Since then, it has become generally accepted that it is the integrated, interactive effects of all steps in the respiratory cascade that help set $\dot{V}_{O_2max}$ (Wagner *et al.* 1997): 'No single step is *the* limiting one, a change in the capacity of anyone step will alter $\dot{V}_{O_2max}$'.

Statement of the problem

This review explores how $\dot{V}_{O_2max}$ is set by structural capacities and functional regulation at each point in the

pathway for oxygen from lungs to skeletal muscle mitochondria. For the periphery, we additionally analyze how the pathways for oxygen and substrates converge at the mitochondria and how substrate fluxes intervene in setting $\dot{V}_{O_2max}$. To study a complex integrated physiological system such as the respiratory cascade, we need to develop an intellectual framework within which we can perform a systems analysis.

To model the respiratory system

If an animal exercises intensely, its muscles elevate its metabolic rate at least tenfold above resting values. A well-trained human or animal athlete doing the same may elevate the rate of O_2 consumption by up to 20-fold above resting values (Fig. 1). In either case, this is accompanied by an increase in cardiac output and heart rate as well as by an increase in minute ventilation and respiratory frequency: O_2 uptake in the lung and O_2 transport by the circulation of blood must be matched to O_2 consumption in the mitochondria of the working muscle cells. At the same time, substrate supply must also be matched to oxidation rate. The basic features of this system are as follows.

(1) Respiration is an integral function involving the coordinated action of all the structures that make up the pathway for O_2 from the lung to the respiratory chain enzymes in tissue mitochondria. Under steady-state conditions, the O_2 flow rate is the same at all levels.

(2) Respiration is a regulated process matched to the instantaneous demands of aerobic metabolism: as muscle ATP consumption increases, O_2 demand is increased proportionally, requiring regulation of the various O_2 and substrate transport functions.

(3) Respiration at the whole-animal level is a limited function. The rate of O_2 consumption can rise to a definable limit, called $\dot{V}_{O_2max}$, beyond which any additional energy must be covered by anaerobic processes. This limit is a characteristic of the individual and is higher in athletes than in untrained subjects; to a certain extent, it is malleable in that it can be elevated by training.

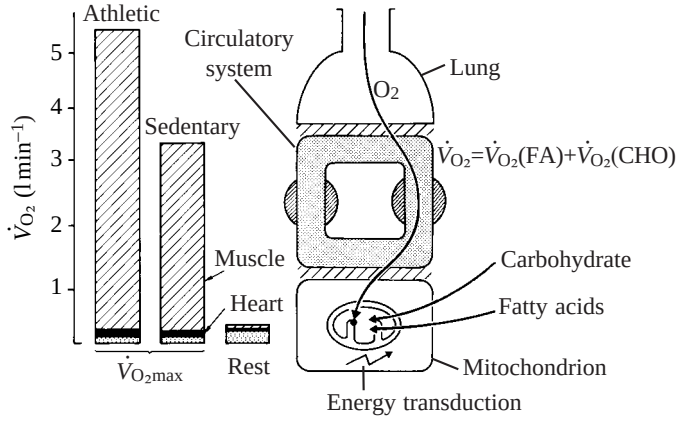


Fig. 1. Model of the respiratory system indicating that the pathways for oxygen and substrates converge on the mitochondria. The histograms to the left show the distribution of $\dot{V}_{O_2}$ to locomotor muscles, heart and other tissues (stippled) under resting conditions and at $\dot{V}_{O_2max}$ in sedentary and endurance-trained humans (after Weibel *et al.* 1991). FA, fatty acids; CHO, carbohydrates.

What sets the limit for aerobic metabolism

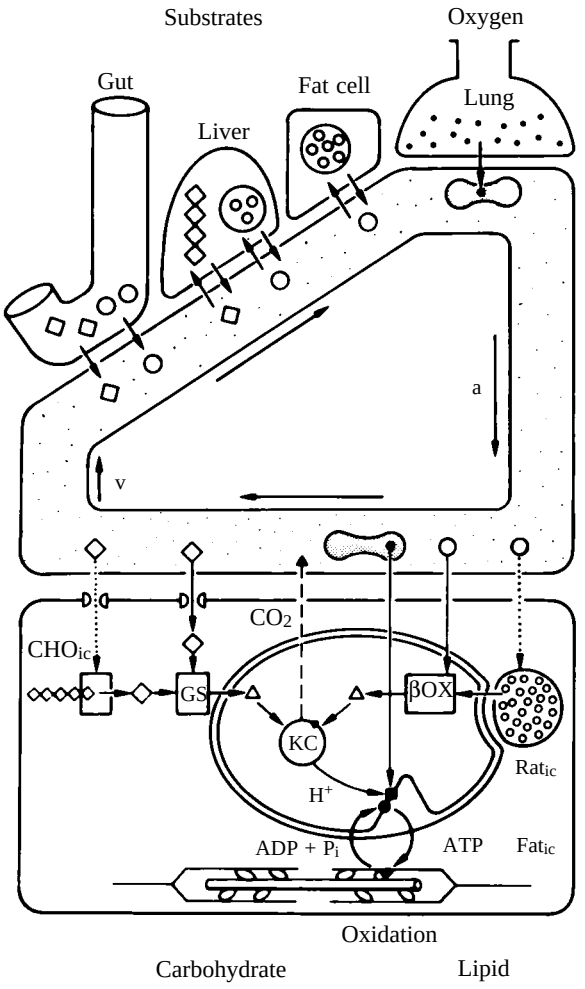
To approach the question of what sets the limit for aerobic metabolism, we must consider the entire pathway for O_2 from the site of uptake in the lung to the sink in the mitochondria (Fig. 2) as well as the pathways for substrates that fuel oxidation from uptake in the gut to oxidation in the mitochondria. The O_2 pathway is simple: O_2 follows a single path without branches.

The substrate pathways are more complex. Both carbohydrate and lipid pathways branch to form four parallel pathways that converge on the mitochondria (Fig. 3). Proteins can be ignored for the purpose of the current analysis because in well-fed subjects their contribution to aerobic metabolism is minimal. During exercise, when rates of substrate oxidation are highest, blood is shunted away from the gut and rates of substrate uptake are lowest. At this point, stores supply most of the fuel for oxidation. Organismic substrate stores outside muscle are in the liver and in the adipose tissue. Inside the muscle cells, carbohydrates are stored as glycogen granules and fatty acids are stored as lipid droplets (Fig. 4). We will have to delineate the importance of the parallel substrate pathways as a function of exercise intensity and duration. We would like to know whether there is a single step in the oxygen or substrate pathway that sets the upper limit to aerobic metabolic rate, or whether the capacities in each of the steps are approximately matched to each other. We would also like to know whether limiting factors are primarily structural or functional.

Structural parameters can limit $\dot{V}_{O_2max}$

Structural parameters intervene at all levels of the pathways. For example, the model in Fig. 2 predicts (a) that the pulmonary diffusing capacity (D_{LO_2}) depends on the surface area available for gas exchange between air and blood, (b) that the stroke volume (Vs) is determined by the size of the heart ventricles, (c) that the O_2 transport capacity of the blood is determined by the erythrocyte volume density, Vv(ec), and (d) that the capacity of cells for oxidative phosphorylation is related to the volume of mitochondria, V(mt).

Structural parameters cannot be regulated or changed on a short time scale because this requires morphogenetic processes. Structure can be modified in response to chronically altered demand. This regulation involves transcriptional activation of structure genes (Puntschart *et al.* 1995). It is well known that, during exercise, cardiac output is immediately regulated by increasing heart frequency (f_H) up to a maximum, with stroke volume essentially unchanged; in contrast, as a consequence of exercise training, heart size increases and maximal cardiac output becomes elevated by a larger stroke



$$\underset{\text{Function}}{\dot{V}_{O_2} = (P_{AO_2} - Pb_{O_2})\{t_C, \Theta_{O_2}\}} \times \underset{\text{Design}}{D_{LO_2}\{S(a), S(c), V(c), Vv(ec), \tau(ht)\}}$$

$$\dot{V}_{O_2} = (\sigma_a Pa_{O_2} - \sigma_{\bar{v}} P\bar{v}_{O_2}) f_H \times V_S\{V(LV)\} \times Vv(ec)$$

$$\dot{V}_{O_2} = (Pb_{O_2} - Pc_{O_2})\{t_C, \Theta_{O_2}\} \times D_{TO_2}\{S(c), V(c), Vv(ec), \delta(c\text{-}mt)\}$$

$$\dot{V}_{O_2} = \left\{ \begin{array}{l} \dot{V}_{O_2}\{\dot{m}_{ATP}\} \times V(mt)\ \{Sv(im,mt)\}\ \text{<muscle>} \\ +\dot{V}_{O_2}\{\dot{m}_{ATP}\} \times V(mt)\ \{Sv(im,mt)\}\ \text{<heart>} \\ +\dot{V}_{O_2}\{\dot{m}_{ATP}\} \times V(mt)\ \{Sv(im,mt)\}\ \text{<other organs>} \end{array} \right\}$$

Fig. 2. Model of the respiratory system expanded to include the pathway for oxygen and the pathways for substrates. The equations to the right express the rate of oxygen flow ($\dot{V}_{O_2}$) as the product of functional and design parameters shown in bold type; parameters that affect these factors are shown within braces {}. The functional parameters include: O_2 partial pressure, P_{O_2}; coefficients of 'haematocrit-specific' O_2 capacitance, σ, which depend on O_2–haemoglobin dissociation; O_2 binding rate, Θ; heart frequency, f_H; capillary transit time, t_C; and mitochondrial O_2 consumption rate as a function of ATP flux, $\dot{V}_{O_2}(\dot{m}_{ATP})$. Design parameters include: diffusion conductance, D, of lung (L) and tissue (T) gas exchangers, which depend on alveolar (A) and capillary (c) exchange surface areas, S(A), S(c); capillary volume, V(c); volume density of erythrocytes, Vv(ec); harmonic barrier thickness, τ(ht); capillary-to-mitochondria diffusion distance, δ(c-mt); mitochondrial volume, V(mt) and inner membrane surface density, Sv(im,mt). a, arterial; v, venous; CHO, carbohydrate; KC, Krebs cycle; βOX, β-oxidation; GS, glycolysis; Vs, stroke volume; V(LV), volume of left ventricle; Pb_{O_2}, capillary P_{O_2}; Pc_{O_2}, intracellular P_{O_2}.

volume at the same maximal heart rate. From this, we conclude that structural parameters set the boundaries within which functional parameters regulate oxygen and substrate fluxes according to the instantaneous demands of the organism. Note that in the example the *actual* functional limitation is heart rate, which has a fixed maximum because stroke volume can be increased by training. So it is both functional and structural parameters that set the limit.

The concept of symmorphosis

The question, therefore, is whether the structural design of the respiratory system is such that it allows the limits to $\dot{V}_{O_2}$ of the sequential steps to be matched to each other and to the overall limit. Common sense dictates that this should be the case, because it does not make sense for the body to build and maintain structures of a fundamental and vital functional system that it will never use. To approach this problem, we have proposed a principle of regulated morphogenesis and of economic design, the hypothesis of 'symmorphosis' (Taylor and Weibel, 1981). Symmorphosis postulates that the design of all components comprising a system is matched quantitatively to functional demand, 'enough but not too much'. The principle of symmorphosis is somewhat akin to the concept of 'homeostasis'. While homeostasis operates in real time on functional variables, symmorphosis operates on structural variables and on different time scales. Symmorphosis is either the consequence of genetic selection

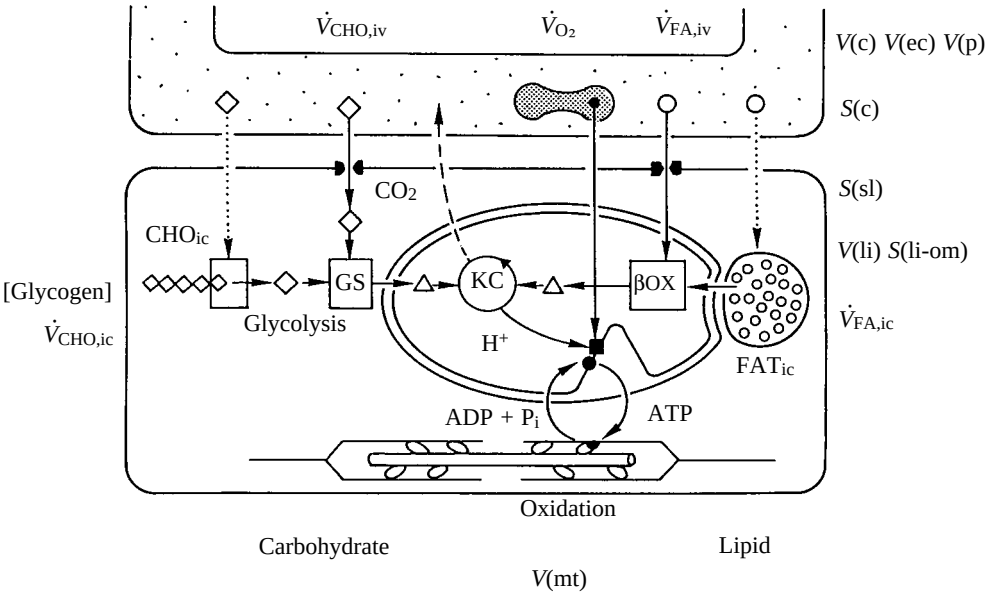


Fig. 3. Model of the structure–function relationship of oxygen and intracellular substrate supply to the mitochondria of skeletal muscle cells. Dots indicate oxygen, open circles indicate fatty acids, squares indicate glucose, the row of squares indicates glycogen and triangles indicate acetyl CoA. The horizontal arrows indicate the pathways of intracellular substrate breakdown from the intracellular stores to the terminal oxidase in the mitochondrial membrane (black square). The vertical arrows indicate the supply routes of oxygen and substrates from the capillaries, with dotted arrows for the supply route to intracellular stores, temporally split from the phase of oxidation (modified from Vock *et al.* 1996*a*). $\dot{V}$, rate of oxidation; FA, fatty acid; CHO, carbohydrate; iv, vascular; ic, intracellular; GS, glycolysis; βOX, β-oxidation; KC, Krebs cycle. Structural variables relevant for transport and metabolism of substrates: *V*, volume; *S*, surface area; c, capillary; ec, erythrocyte; p, plasma; sl, sarcolemma; li, lipid; li-om, lipid-to-mitochondria interface; mt, mitochondria.

or it can be brought about by epigenetic structural malleability.

In a pragmatic approach, we can test the hypothesis of symmorphosis by taking advantage of the fact that $\dot{V}_{O_2max}$ varies among individuals and species. We can study the variation of structural and functional parameters in relation to the variation in $\dot{V}_{O_2max}$. By comparing the differences in structural and functional variables with the variation in $\dot{V}_{O_2max}$, we attempt to identify parameters that vary directly in proportion to $\dot{V}_{O_2max}$ and whose ratio to $\dot{V}_{O_2max}$ is, accordingly, invariant.

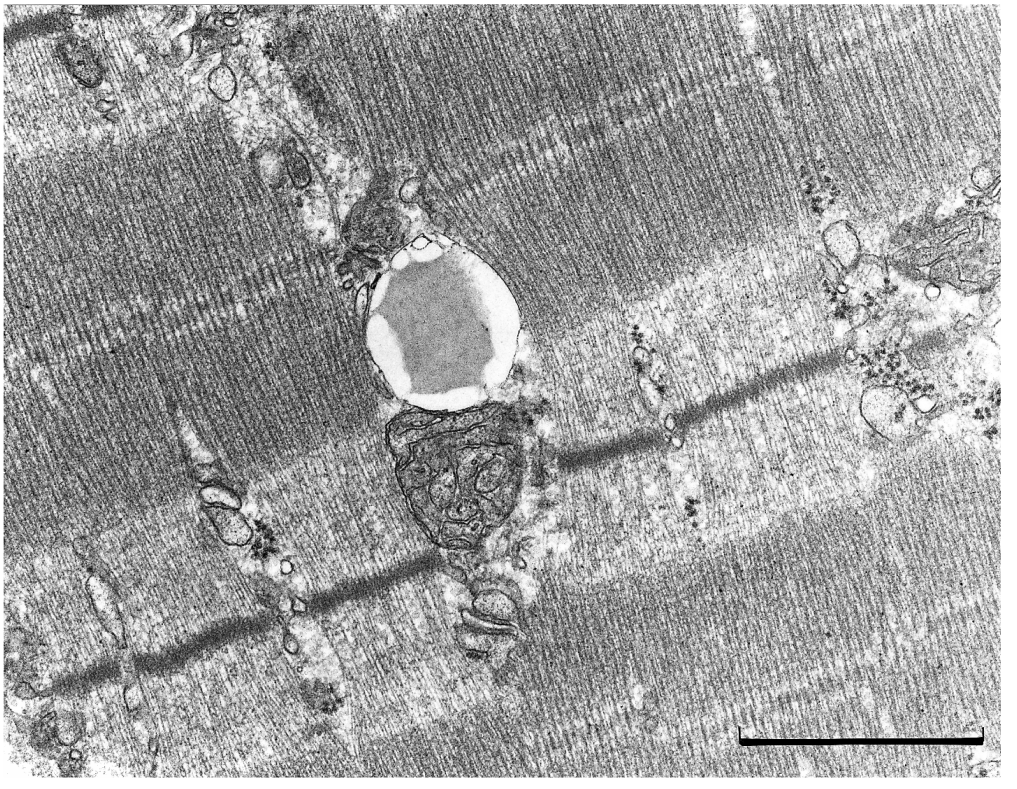


Fig. 4. Electron micrograph of a longitudinal section of skeletal muscle tissue. In the centre, at the level of the Z-line, we find an interfibrillar mitochondrion with a lipid droplet immediately adjacent. Glycogen granules (small black dots) can be seen preferentially in the region of the A-band. Scale bar, 1 μm.

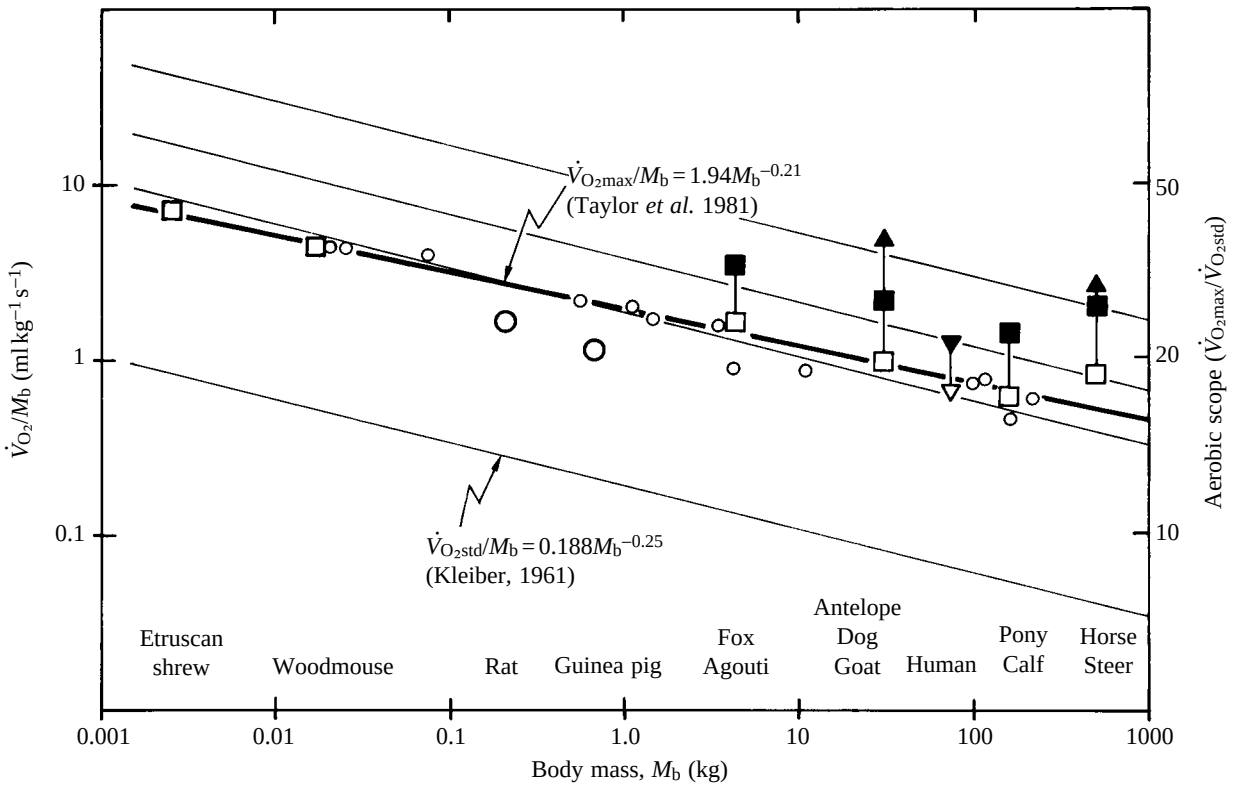


Fig. 5. Allometric plot of mass-specific rate of O_2 consumption $\dot{V}_{O_2}$, showing Kleiber's curve (1961) for standard (basal) $\dot{V}_{O_2}/M_b$ isopleths for theoretical aerobic scope (fine lines) and the allometric plot for $\dot{V}_{O_2max}/M_b$ (open circles, heavy line). Adaptive pairs are shown as open and filled squares for sedentary and athletic species, respectively; filled upright triangles show the highest values measured in these size classes on Thoroughbred racehorses and pronghorn antelope. Human $\dot{V}_{O_2max}$ data are for sedentary people and highly trained athletes (open and filled triangle, respectively). The graph is modified from Weibel *et al.* (1992). $\dot{V}_{O_2std}$, standard metabolic rate. M_b, body mass.

The study of 'limiting factors' using comparative physiology

Ideally, one would like to change $\dot{V}_{O_2max}$ in a single individual, e.g. by exercise training or by cold exposure. We call these changes, which reflect the malleability of the respiratory system, *induced variation*. The problem with induced variation is that only relatively small differences in $\dot{V}_{O_2max}$ can be induced experimentally (typically of the order of 15 %, approaching the limits of the resolution of the structural measurements). This type of experiment is therefore not well suited for a systems analysis. Larger variations in $\dot{V}_{O_2max}$ are observed among mammalian species as a consequence of two types of selection pressure during evolution.

(1) *Allometric variation.* Metabolic requirements are related to body mass (M_b) in such a way that small animals have higher resting and maximal oxygen consumption rates than large ones. These differences are usually expressed as power functions that describe the change of any functional or structural parameter as body mass changes, $\dot{V}_{O_2max}$ is found to be proportional to $M_b^{0.8}$; accordingly, $\dot{V}_{O_2max}/M_b > M_b^{-0.2}$ (Fig. 5). Allometric variation accounts for enormous differences in $\dot{V}_{O_2max}/M_b$: a mouse consumes approximately six times more O_2 than a cow per unit body mass (Weibel *et al.* 1981*b*).

(2) *Adaptive variation*. In several size classes, nature has selected species for athletic performance (Fig. 5). Thus, we find that nature's athletes such as horses and dogs have a $\dot{V}_{O_2max}$ that is more than twice that of sedentary species of similar size such as cattle and goats (see Table 1) (Taylor *et al.* 1987; Jones *et al.* 1989), and super-athletes such as the pronghorn antelope achieve even higher values.

Comparative studies using allometric and adaptive variations in $\dot{V}_{O_2max}$ have proved invaluable for a fundamental understanding of the design of the mammalian respiratory system and its functional limits. Not only do they provide us with large differences in $\dot{V}_{O_2max}$, and hence with an excellent signal-to-noise ratio for our experiments, but they also offer the advantage that it is possible to sample tissue from all steps of the respiratory cascade. This enables us to sample tissue for morphometric quantification of the functionally relevant structural parameters on all levels. We will exploit the comparative data to generate a perspective of the fundamental design principle of the respiratory system in mammals.

Muscle mitochondria set the demand for oxygen

When mammals exercise at their maximal rate of oxygen consumption, the mitochondria located in their skeletal

muscles consume more than 90 % of the available oxygen and substrates (Fig. 2; Mitchell and Blomqvist, 1971). We are therefore justified in concentrating on these particular mitochondria in our search for invariant design parameters in the respiratory system. What is the relevant structural parameter of skeletal muscle mitochondria to relate to the large variation in $\dot{V}_{O_2max}/M_b$ in allometric and adaptive variation? It has been shown that the volume of mitochondria in skeletal muscle is a good measure of the quantity of matrix enzymes as well as of the quantity of respiratory chain enzymes present and, therefore, it is also an appropriate structural parameter to relate to $\dot{V}_{O_2max}$. This is the case when comparing mammals; in hummingbirds, reptiles and insects, we may have to use other structural variables with which to relate the rate of oxygen consumption (Suarez, 1992).

The maximal rate of oxygen consumption by mammalian mitochondria can then be expressed as the product of mitochondrial volume, $V(mt)$, and a functional parameter:

$$\dot{V}_{O_2max}/M_b = V(mt)/M_b \times \dot{V}_{O_2}(mt), \quad (1)$$

where $\dot{V}_{O_2}(mt)$ is the actual rate at which mitochondria consume oxygen.

How do these parameters change as $\dot{V}_{O_2max}/M_b$ is varied by allometric, adaptive or induced variation? Because time is the fundamental variable in allometric variation (Lindstedt and Calder, 1981; Schmidt-Nielsen, 1984), we would anticipate *a priori* that $\dot{V}_{O_2}(mt)$ would vary directly with $\dot{V}_{O_2max}/M_b$, being six times greater in a mouse than a cow, whereas $V(mt)/M_b$ should be invariant. This would seem to be an eminently reasonable design principle because the relative volume of muscle fibres occupied by mitochondria would not increase with demand. This is because relative muscle volume, $V(musc)/M_b$, follows the general allometric rule of organ size and is invariant with body size. On average, skeletal muscles make up some 40–45 % of the total body mass (Schmidt-Nielsen, 1984). If $V(mt)/M_b$ changed with $\dot{V}_{O_2max}/M_b$, the contractile machinery would become progressively more diluted as demand increased; this might pose a serious problem for small animals in which mitochondria could occupy a significant fraction of the cell volume.

Contrary to our expectations, small animals have more mitochondria in each gram of muscle than do large animals. Quantitative measurements of mitochondrial volume in animals spanning a range of body masses from less than 20 g (woodmice; Hoppeler *et al.* 1984) to over 500 kg (steers and horses; Hoppeler *et al.* 1987*a*) reveal that the mitochondrial volume of skeletal muscles varies almost directly with $\dot{V}_{O_2max}$, whereas $\dot{V}_{O_2}(mt)$ of the unit mitochondrial volume does not change with size. Compared with the cow, the mouse has six times the volume of mitochondria in its skeletal muscles so that, at $\dot{V}_{O_2max}$, each millilitre of mitochondria in both cow and mouse would consume, on average, 4–5 ml O_2 ml^{-1} min^{-1} (Fig. 6). Thus, the five- to tenfold difference in $\dot{V}_{O_2max}$ is matched by corresponding differences in the amount of mitochondria that animals make, whereas the functional parameter, the rate at which each unit of structure consumes oxygen, is the same, irrespective of size.

Why is the widespread fundamental design principle that time

Table 1. *Differences in morphometric and physiological parameters of muscle mitochondria and capillaries and of heart, blood and lungs with adaptive variation of* $\dot{V}_{O_2max}$ *in three species pairs*

Design	Body	Mitochondria		Blood	Capillaries			Heart		Lung	
		$V(mt)/M_b$	$\dot{V}(mt)$	$Vv(ec)$	$V(c)/M_b$	$V(c)\dot{\times}Vv(ec)$		Vs/M_b	$Vs\dot{\times}Vv(ec)$	D_{LO_2}/M_b	$\dot{D}_{LO_2}$
Function	$\dot{V}_{O_2max}/M_b$		$\dot{V}_{O_2max}$			$\dot{V}_{O_2max}$	f_H		$\dot{V}_{O_2max}/f_H$		$\dot{V}_{O_2max}$
Units	($ml\,kg^{-1}\,s^{-1}$)	($ml\,kg^{-1}$)	($ml\,ml^{-1}\,s$)		($ml\,kg^{-1}$)	($ml\,ml^{-1}\,s$)	(min^{-1})	($ml\,kg^{-1}$)	($ml\ ml^{-1}$)	($ml\,mmHg^{-1}$ $kg^{-1}\,s^{-1}$)	(mmHg)
25–30 kg											
Dog	2.29	40.6	17.7	0.50	8.2	1.79	274	3.17	3.16	0.118	0.052
Goat	0.95	13.8	14.5	0.30	4.5	1.42	268	2.07	2.92	0.080	0.084
Dog/goat	2.4*	2.9*	1.2	1.68*	1.8*	1.26	1.02	1.53*	1.08	1.48*	0.61*
150 kg											
Pony	1.48	19.5	13.2	0.42	5.1	1.45	215	2.50	2.54	0.079	0.053
Calf	0.61	9.2	15.1	0.31	3.2	1.63	213	1.78	3.21	0.050	0.082
Pony/calf	2.4*	2.13*	0.9	1.35*	1.6*	0.89	1.02	1.40*	0.79	1.57*	0.65*
450 kg											
Horse	2.23	30.0	13.5	0.55	8.3	2.05	202	3.11	2.58	0.108	0.048
Steer	0.85	11.6	13.7	0.40	5.3	2.49	216	1.52	2.58	0.054	0.064
Horse/steer	2.6*	2.6*	1.0	1.4*	1.6*	0.82	0.94	2.1*	1.00	2.0*	0.76*
Athletic/sedentary	2.5*	2.5*	1.03	1.5*	1.7*	0.99	1.0	1.7*	0.96	1.7*	0.67*

Modified from Weibel et al. (1991). The last line presents overall ratios for athletic/sedentary species.
Asterisks denote ratios significantly different from 1.
The abbreviations are defined in the text and the legends to the figures.

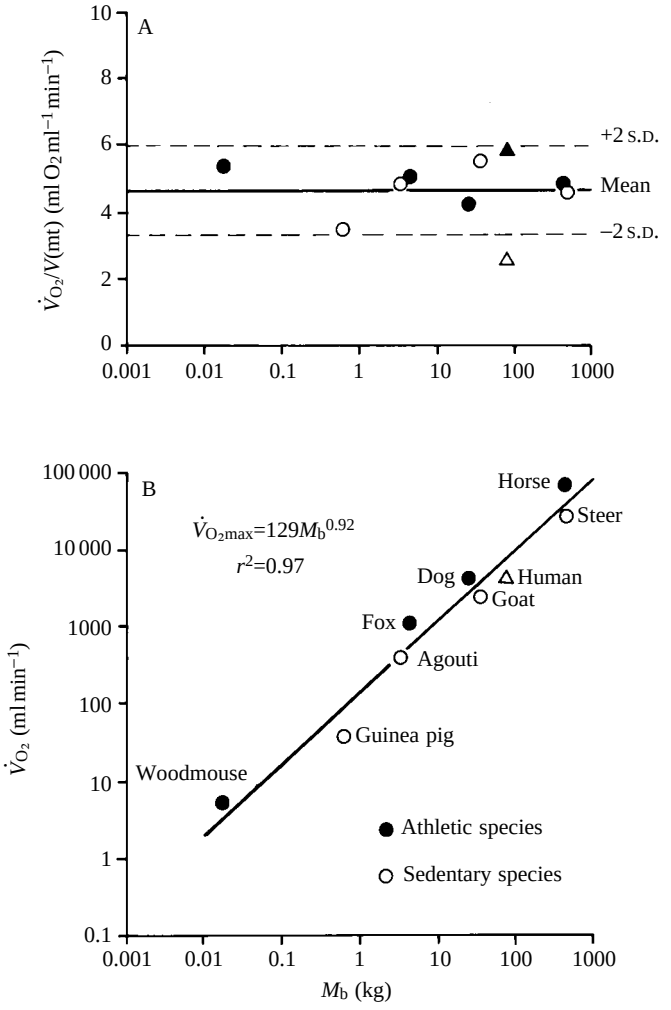


Fig. 6. Allometric plot of $\dot{V}_{O_2}$ per mitochondrial volume, V(mt) (A) and whole-body $\dot{V}_{O_2}$ (B) for species ranging in mass from 16 g to 450 kg. The open triangles indicate data for humans considering total muscle mass and 'active' muscle mass (filled triangle). M_b, body mass.

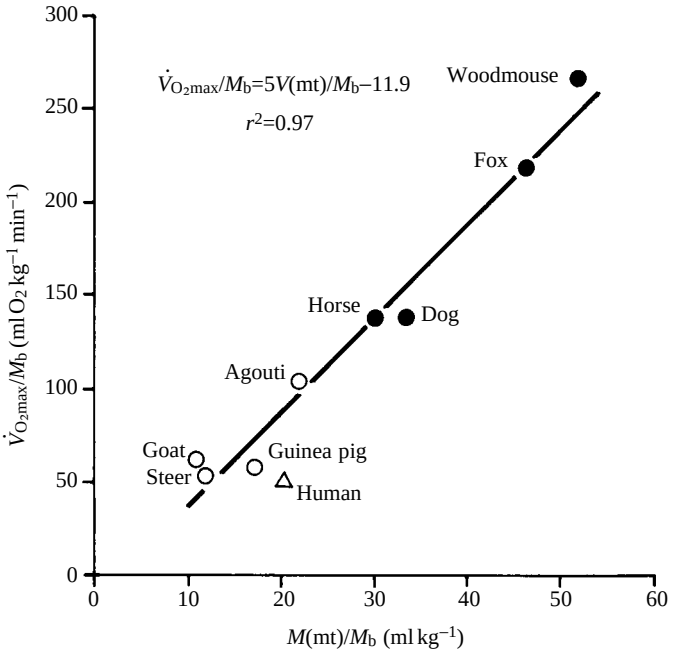


Fig. 7. Body-mass-specific $\dot{V}_{O_2max}$ plotted as a function of body-mass-specific mitochondrial volume, V(mt), of skeletal muscle mitochondria for animals differing over a fivefold range of $\dot{V}_{O_2max}$. The open triangle indicates data calculated for the entire musculature of humans. It can be seen that humans have 'excess' mitochondrial volume in comparison with their $\dot{V}_{O_2max}/M_b$. This discrepancy disappears when active muscle mass is considered. M_b, body mass.

constants vary with body size violated with respect to mitochondria? A possible explanation may lie in their origin and their genetics. The enzyme systems of mitochondria and their spatial organization within membranes are very similar to those found in bacteria. So similar, in fact, that it has been suggested that mitochondria evolved from bacteria that were incorporated into the first eukaryotes in an endosymbiotic relationship (Margulis, 1981). This is supported by the finding that mitochondria contain their own DNA and ribosomes, which are both similar to those of bacteria. If we consider mitochondria as endosymbiotic bacteria that respond to increased energy demand by reproducing, then their individual size and composition should not change, and there is no reason to expect that the activities of their enzymes will change with the O_2 demand of the host cell.

If we now consider adaptive variation, we find that mitochondria have adapted to the 2.5-fold differences in aerobic capacity among animals of the same size in the same way that mitochondria adapted to differences with allometry (Hoppeler *et al.* 1987*b*; Mathieu *et al.* 1981). The higher demand is met simply by building more of the same structure. The 2.5-fold greater $\dot{V}_{O_2max}/M_b$ of dogs, ponies and horses compared with that of goats, calves and steers is matched by a 2.5-fold larger total volume of mitochondria in their muscles, whereas the average rate at which each millilitre of mitochondria consumes oxygen is again the same, namely, 4–5 ml O_2 ml^{-1} min^{-1} (Table 1).

Overall, the comparative approach has revealed that the maximal rate of O_2 consumption by skeletal muscle mitochondria is invariant at $\dot{V}_{O_2}$(mt)=4–5 ml O_2 ml^{-1} min^{-1} and, accordingly, that V(mt)/$\dot{V}_{O_2max}$=0.2 ml O_2 ml^{-1} min^{-1} and is invariant under all circumstances.

In this context, humans are different. Because of bipedal locomotion, they normally reach $\dot{V}_{O_2max}$ while performing exercises that do not involve all of their muscles, as is the case for quadrupedal animals. As a consequence, apparent $\dot{V}_{O_2}$(mt) is only half of what we find in animals (Fig. 7). Alternatively, this has been interpreted as humans having 'excess' muscle oxidative capacity (Gollnick and Saltin, 1982).

In summary, we can conclude that there is a good match between structure and function in the design of the mammalian respiratory system at the level of the mitochondria with allometric and adaptive variations in $\dot{V}_{O_2max}$. In both cases, the differences in maximal rates of oxygen consumption are

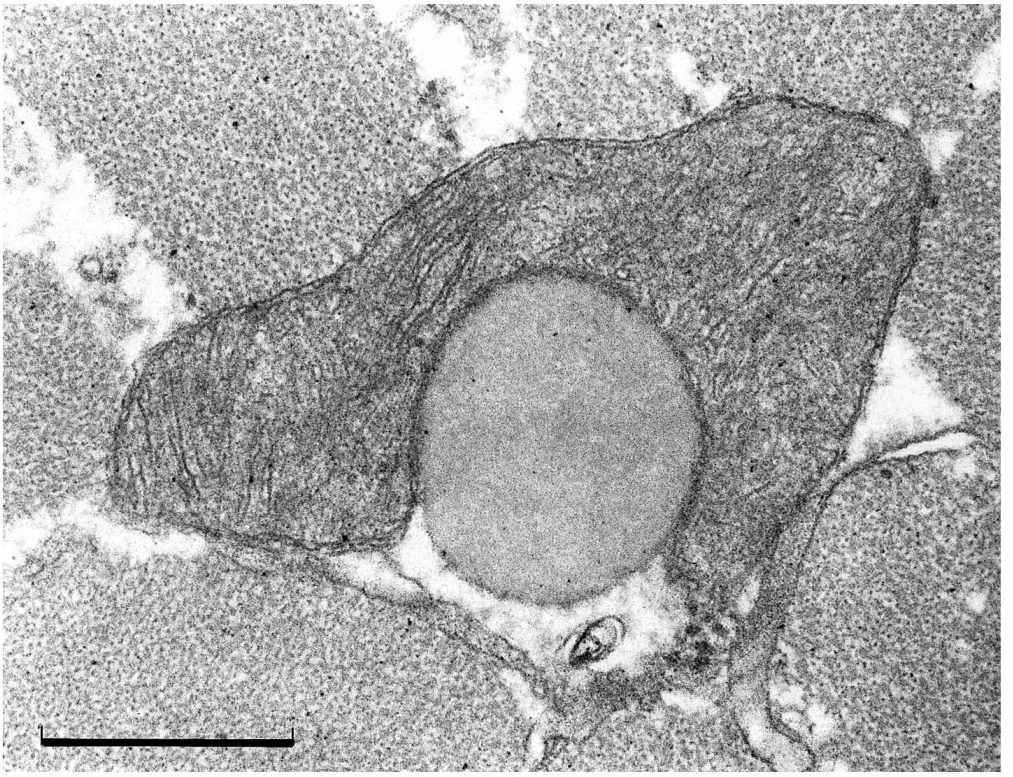


Fig. 8. Electron micrograph of a cross section of a portion of a muscle fibre. The intimate contact between the mitochondrial outer membrane and the lipid droplet can be clearly seen. Scale bar, 0.5 μm.

matched by corresponding differences in the amount of mitochondrial structure for both oxygen and substrates, whereas the average rate at which each unit of structure consumes oxygen is invariant.

The supply of substrates from cellular stores to mitochondria

When exercising at $\dot{V}_{O_2max}$, substrates must be transported into the mitochondria at a rate sufficient to fuel oxidative phosphorylation. The oxidation of 1 mol of glucose consumes 6 mol of oxygen, and 1 mol of fat consumes 23 mol of O_2. It has been clearly established in dogs and goats that circulating carbohydrates and lipids can supply only a fraction of the total substrate demand of mitochondria in working muscles (Weber *et al.* 1996*a*,*b*). Both carbohydrate and lipid transport from capillaries into muscle cells appear to be maximal at quite low exercise intensities corresponding to approximately 40 % of $\dot{V}_{O_2max}$ and cannot be upregulated at higher work intensities. Instead, the mitochondria depend on obtaining their fuels from intracellular substrate stores so that, at $\dot{V}_{O_2max}$, over 80 % of the fuel is supplied from glycogen granules. These results were obtained in a comparative setting of adaptive variation, but they are very similar to the situation observed in exercising humans, where a heavy reliance on intracellular substrate stores at higher work intensities has also been demonstrated (Romijn *et al.* 1993). From this, it follows that substrates for mitochondrial oxidation at work intensities of approximately 80 % of $\dot{V}_{O_2max}$ must primarily be supplied from glycogen granules and lipid droplets inside the muscle cells, with no more than 20–30 % of the fuel coming from the capillaries. There is strong evidence that this is due to a limitation of fuel supply from the capillary, with the sarcolemma acting as the main barrier (Vock *et al.* 1996*a*,*b*).

It is currently not clear what throttles triacylglycerol oxidation in exercising muscle. Is it the transfer of fatty acids to the mitochondrial matrix or is it β-oxidation? In contrast, the rate at which glycogen stores can supply pyruvate to the Krebs cycle is not limited: they can supply it up to the limit of oxidation rate by the mitochondria and can then achieve a further several-fold increase in order to fuel anaerobic glycolysis. Clearly, therefore, oxidation in the mitochondria is not limited by the supply of fuel but rather by the supply of oxygen from the capillaries – or by the quantity of mitochondria that can perform oxidative phosphorylation.

Microcirculatory supply of oxygen and substrates

Oxygen diffuses from the capillaries to the mitochondria, and the flow of oxygen at this step can be described as the product of a conductance and a pressure head (Fig. 2):

$$\dot{V}_{O_2max} = D_{TO_2} \times (P_{bO_2} \times P_{cO_2}), \quad (2)$$

where P_{bO_2} is the mean capillary P_{O_2} and P_{cO_2} is the mean intracellular P_{O_2}. The conductance, D_{TO_2}, extends from the erythrocytes in the capillaries to the mitochondrial oxygen sinks. We do not, as yet, have a model or set of measurements that allows us to formulate the dependence of D_{TO_2} on

functional and structural variables. What can we use as a relevant structural parameter to relate oxygen flow at this step in the respiratory system to the variations in $\dot{V}_{O_2max}$?

In general terms, the maximal conductance must be related to the volume of capillaries in skeletal muscles, $V(c)$, a structural parameter that has been measured. $V(c)$ is directly proportional to the capillary surface area available for diffusion of oxygen out of the blood, since the diameter of capillaries does not change with either size or adaptation. $V(c)$ also plays an important role in determining the time available for diffusion as blood transits the capillary network.

Using $V(c)/M_b$ as the structural parameter, we can express the maximal flow of oxygen through this step in the respiratory system in the same way in which we have considered oxygen consumption by the mitochondria, expressed as the product of $V(c)/M_b$ and a functional parameter, $\dot{V}_{O_2}(c)$, the rate at which oxygen diffuses out of each unit volume of capillaries

$$\dot{V}_{O_2max}/M_b = [V(c)/M_b] \times \dot{V}_{O_2}(c) . \quad (3)$$

We would expect these parameters to change with variations in $\dot{V}_{O_2max}/M_b$ in parallel with the structural and functional parameters of the mitochondria that set the demand. What do we find?

With allometric variation, small animals have more capillaries in each gram of their muscles than do large animals. When quantitative measurements of capillary volume are made on the same individuals for which mitochondrial volume measurements were made, one finds that average mass-specific capillary volume, like mitochondrial volume, decreases in direct proportion to $\dot{V}_{O_2max}/M_b$ over the size range from 20 g (mice) to 500 kg (horses), whereas the rate of oxygen delivery per millilitre of capillary, $\dot{V}_{O_2}(c)$, is nearly the same over the entire size range of animals (Taylor *et al.* 1989). Thus, we can conclude that $\dot{V}_{O_2max}/V(c)$ is invariant with size and has a value of $15\,ml\,O_2\,ml^{-1}\,min^{-1}$.

This constant value for $\dot{V}_{O_2}(c)$, like that for $\dot{V}_{O_2}(mt)$, is a minimal value which assumes that, at $\dot{V}_{O_2max}$, all of the capillaries are utilized and that oxygen diffuses out of all of them at the same rate. Irrespective of whether this is the case, it clearly indicates that the capillary surface area available for diffusion increases directly with the rate of diffusion of oxygen out of the capillaries over the five- to tenfold variation in $\dot{V}_{O_2max}/M_b$ with size. We can see that the ratio $V(c)/V(mt)$ must also be invariant (i.e. approximately 0.3 ml of capillaries for each ml of mitochondria), since both $\dot{V}_{O_2max}/V(mt)$ and $\dot{V}_{O_2max}/V(c)$ are invariant with size (Hoppeler *et al.* 1981).

With adaptive variation, we find a different pattern of adjustment of capillaries to oxygen demand (Conley *et al.* 1987). $V(c)/M_b$ changes by only 1.7-fold with the 2.5-fold difference in $\dot{V}_{O_2max}/M_b$ between the dog/goat, pony/calf and horse/steer pairs (Table 1). Using equation 3, we can calculate that oxygen diffuses out of each millilitre of capillary (each square centimetre of its surface) 1.5 times faster in the more aerobic species. Part of the explanation for this higher rate of diffusion lies in a 1.6-fold higher oxygen concentration in the arterial blood entering the capillary and a corresponding 1.6-fold greater extraction of oxygen as the blood transits the capillary bed. This results from a 1.5-fold higher haemoglobin concentration in the blood of the athletic species, which is due to a higher haematocrit or erythrocyte

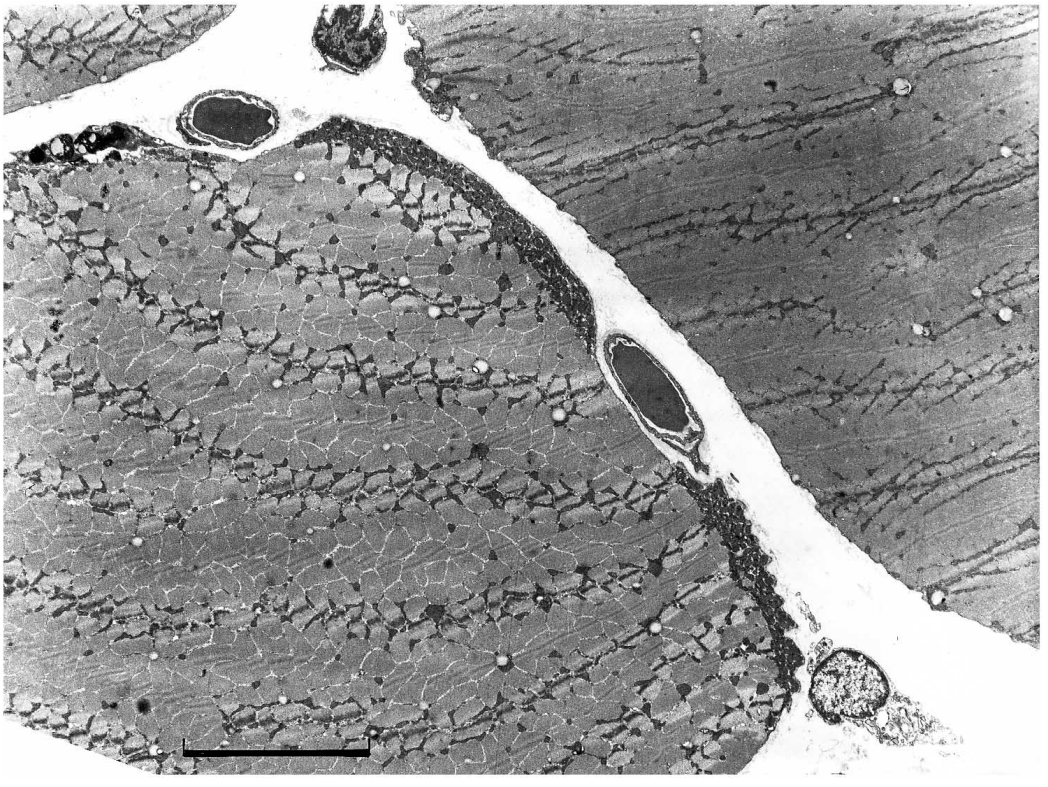


Fig. 9. Electron micrograph of portions of human skeletal muscle fibres in cross section. Capillaries containing erythrocytes are found between muscle fibres. Subsarcolemmal mitochondria are massed in the periphery of one of the muscle fibres, but not immediately between myofibrils and capillaries. Scale bar, 10 μm.

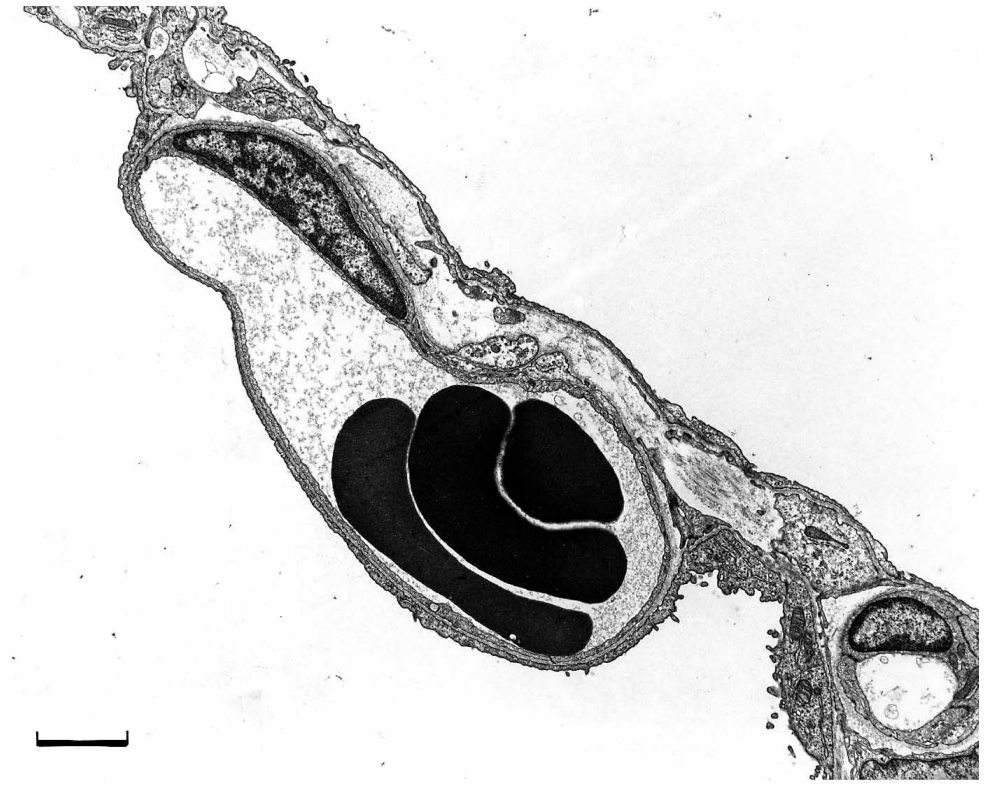


Fig. 10. Electron micrograph of the alveolar septum containing a capillary with three erythrocytes. Note the extremely thin tissue barrier between the alveolar air and the erythrocytes. Scale bar, 2 μm.

concentration, *V*v(ec). Multiplying the 1.7-fold greater volume of capillaries by the 1.6-fold greater extraction of oxygen across the capillary gives a 2.7-fold greater oxygen delivery during the transit of the blood through the capillary bed.

We can thus conclude that there is a reasonably good match between structure and the oxygen supply function at the level of the capillaries. In the case of allometric variations in $\dot{V}_{O_2max}/M_b$, the higher rates of oxygen consumption are matched by corresponding differences in the amount of capillary structure, whereas the rate at which O_2 diffuses from each square centimetre of capillary surface is invariant in this case. The design of the capillaries at this level is closely matched to that of the mitochondria that consume the oxygen. In adaptive variation, capillaries are incompletely matched, but this is compensated by a higher haemoglobin or erythrocyte concentration. For both allometric and adaptive variation, it therefore appears that the mass of haemoglobin in capillaries is matched to the mass of mitochondria; this is achieved by varying capillary volume and haemoglobin concentration. The invariant ratio of structural parameters, therefore, is expressed as: $V(ec)/V(mt) \approx M(Hb)/V(mt) = 4\times10^{-2}\,g\,ml^{-1}$, where M(Hb) is mass of hemoglobin and V(ec) is erythrocyte volume. Consequently, we find that the invariant ratio to $\dot{V}_{O_2max}$ is expressed as: $V(c) \approx Vv(ec)/\dot{V}_{O_2max} = 1.8\,ml\,ml^{-1}\,O_2\,s^{-1}$), where *V*v(ec) is the volume density of erythrocytes, and thus involves two structural variables, i.e. capillary volume V(c) and erythrocyte concentration in the blood.

Reviewing the transfer processes for oxygen and substrates from capillaries to myocytes, we can conclude that the muscle microvasculature is optimally designed for the transfer of oxygen, but not for the transfer of substrates. Because of the lack of substantial oxygen stores, oxygen has to be supplied by the circulation at a rate to match the demand of the contracting muscle cells. In contrast, only a limited quantity of substrates is supplied from the vasculature during exercise. Animals and humans working at intensities above 30 % of $\dot{V}_{O_2max}$ rely increasingly on intracellular substrate stores, mainly on glycogen, at high work intensities. These stores will eventually be exhausted during exercise, leading to muscle fatigue. The intracellular stores can then be replenished during periods of rest at low transfer rates.

Convective transport of oxygen and substrates by the heart

Oxygen and substrates are transported convectively from the capillaries in the lung to the capillaries in the muscle by the circulation. Delivery of oxygen by the circulation depends on the properties of the heart as the pump and on those of the blood as the carrier, and both can be varied to adjust to differences in demand. Oxygen flow at this step can be described as the product of the maximal cardiac output, $\dot{Q}_{max}/M_b$, and the arteriovenous oxygen concentration difference, $Ca_{O_2}-Cv_{O_2}$ (Fig. 2):

$$\dot{V}_{O_2max}/M_b = (\dot{Q}_{max}/M_b) \times (Ca_{O_2} - Cv_{O_2}) . \quad (4)$$

The relevant structural parameter of the pump is obviously the size of the heart, which determines the amount of blood pumped with each contraction, the stroke volume *V*s. The flow of blood

at this step is the product of V_s/M_b and a functional parameter, f_H, the maximal frequency of contraction of the pump:

$$\dot{Q}_{max}/M_b = f_H \times V_s/M_b . \quad (5)$$

$C_{a_{O_2}}-C_{v_{O_2}}$ will depend on a second structural parameter, i.e. the amount of haemoglobin or erythrocytes contained in the blood.

In allometric variation, we would anticipate that time (i.e. f_H) would vary with size, while the structural parameters, haemoglobin concentration and V_s/M_b, would be invariant. What information we have supports this idea. On average, the heart makes up the same fraction of body mass over the size range of mammals from mice to cows, approximately 0.58 % (Prothero, 1979); likewise, haemoglobin concentration and the oxygen-carrying capacity of the blood do not vary systematically with body size. Mammals spanning a range of body size from bats to horses have, on average, approximately 13 g of haemoglobin per 100 ml of blood, which can carry 17.5 ml of oxygen (Schmidt-Nielsen, 1984). The invariant heart size and O_2-carrying capacity of the blood suggest that body-size-dependent differences in circulatory transport are brought about entirely by increases in heart frequency.

The structural and functional variations in circulatory transport of oxygen with adaptive variation are very clear-cut. Here, the animals follow the general principles of design: f_H is determined by size, and structures vary with O_2 demand. Maximal heart frequencies of goats and dogs, ponies and calves, and horses and steers are nearly identical pairwise for animals of the same size, despite 1.4- to 2.5-fold difference in $\dot{V}_{O_2max}/M_b$ (Jones *et al.* 1989; Karas *et al.* 1987*b*). The structural parameters V_s/M_b and haemoglobin concentration account for all of the 2.5-fold difference in oxygen delivery at this step. These studies indicate that these animals are operating at or close to the upper limit of their structural capacity for convective transport of oxygen in the circulatory system at $\dot{V}_{O_2max}$ (Taylor *et al.* 1987). The available structures (stroke volume and haemoglobin concentration) for both $\dot{Q}_{max}$ and $C_{a_{O_2}}-C_{v_{O_2}}$ appear to be fully exploited by the time an animal has increased its rate of oxygen consumption from resting to maximal levels. Thus, at this step of O_2 transport in the respiratory system, there appears to be a match between maximal rates of O_2 delivery and the structures involved, a finding that is in accord with the predictions of symmorphosis.

The limited information we have therefore suggests that the structural parameters V_s/M_b and V_v(ec) are invariant in allometric variation, but variant in adaptive variation, whereas the functional parameter f_H is invariant in adaptive variation, but variant in allometric variation.

In summary, while maximal heart rate is important for modulating cardiac output with allometric variation of body mass, in adaptive as well as in induced variation, stroke volume is the main factor determining cardiac output in mammals of a given body size. An important additional component is haematocrit, which is found to be larger in athletic animals.

Oxygen diffusion in the lung

The transfer of O_2 from the air to the blood in the lung is achieved by diffusion. The O_2 flow rate is determined by the product of the partial pressure difference as driving force and the conductance of the gas exchanger (Bohr, 1909), such that (see Fig. 2):

$$\dot{V}_{O_2} = D_{L_{O_2}} \times (P_{A_{O_2}} - P_{b_{O_2}}) . \quad (6)$$

The partial pressure difference between alveolar air and capillary blood ($P_{A_{O_2}}-P_{b_{O_2}}$) is a functional variable that essentially depends on (a) the ventilation of alveoli through the airways and (b) the perfusion of capillaries by the circulation. In contrast, the diffusion conductance for O_2, the diffusing capacity ($D_{L_{O_2}}$), is largely determined by the following structural parameters (see Fig. 2): the alveolar and capillary surface areas [S(A) and S(C)], the harmonic mean barrier thickness of the tissue and of the plasma layer separating the erythrocytes from the endothelium, τ(ht), and the capillary blood volume, V(C).

The morphometric parameters entering the calculation of $D_{L_{O_2}}$ (see Weibel, 1997) are essentially determined by two variables: the lung volume, V(L), and the size or density of the 'building blocks' of the gas-exchange units in the lung parenchyma. The ultimate building block of the gas exchanger is the alveolar septum, with morphometric characteristics being the fraction of septum occupied by capillaries, the capillary volume per septal (alveolar) surface area, V(C)/S(A), the density of erythrocytes or the haematocrit and the harmonic mean thickness of the tissue barrier (Fig. 10). These septa are built into the acinus as alveolar walls in the form of a three-dimensional maze; accordingly, the alveolar surface density, S_v(A), is a measure of the building-block characteristics of lung parenchyma. This hierarchical design provides several options for varying diffusing capacity. Thus, the total alveolar surface area, S(A), is the product of V(L) and the alveolar surface density, S_v(A); furthermore, capillary volume is the product of V(C)/S(A) and S(A). To increase $D_{L_{O_2}}$, the lung would have to increase, for example, the alveolar surface area, and this can be achieved either by increasing lung volume or by increasing the alveolar surface density by packing more alveolar septa into the unit volume of lung parenchyma. Alternatively or additionally, the loading of capillaries onto the septum could be increased or the barrier thickness could be decreased.

The first questions with respect to the design of the gas exchanger are which of these options are used, or whether any of these basic design parameters are invariant with allometric and adaptive variation in $\dot{V}_{O_2max}$.

Let us first consider lung volume. The general idea is that mass-specific lung volume is invariant with body size. At closer inspection, we find, however, that V(L) increases slightly (but significantly) as $M_b^{1.06}$ (Gehr *et al.* 1981), with the result that V(L)/M_b varies from 35 ml kg^{-1} in shrews and mice to 60 ml kg^{-1} in dog, man and cow and can even reach 100 ml kg^{-1} in the horse. This last value indicates that, in adaptive variation, relative lung volume is an important variable, being larger in the athletic species (Weibel *et al.* 1987; Constantinopol *et al.*

1989). In a study of the pronghorn antelope, whose $\dot{V}_{O_2max}$ is twice that of the dog, we found the increase in lung volume (to nearly 5 l for a heavy 20 kg animal!) to account for most of the adaptive lung change (Lindstedt *et al.* 1991).

Among the building-block characteristics, the parameters that characterize septum structure are invariant in adaptive variation (Weibel *et al.* 1987; Constantinopol *et al.* 1989), but they show a weak allometric variability (Gehr *et al.* 1981). The packing of alveolar septa into lung parenchyma, measured by $S_V(A)$ (which is inversely proportional to alveolar diameter), appears to be invariant in the size range of animals between 1 and 100 kg (Weibel *et al.* 1981*a*), assuming values of 400–500 cm^{-1} irrespective of whether the animals are athletic or sedentary. However, it increases drastically in small mammals, up to 1500 cm^{-1} in the shrews, and it falls to 250 cm^{-1} in large mammals so that, over the entire mammalian size range, we find that $S_V(A)$ decreases as $M_b^{-0.11}$.

Quite evidently, all these parameters are subject to a number of constraints. The lung volume is limited by the space available in the chest cavity. The packing of alveolar septa into the air space is limited by the requirements for adequate ventilation, as well as by mechanical constraints related to surface tension. The smaller the alveoli, the greater the surface forces; and the larger the alveoli, the more costly is alveolar ventilation. Mammals may have indeed found an optimum range for the size of these building blocks from which they deviate only in the very small species and perhaps in the largest, but to a lesser extent.

How are these building blocks related to O_2 uptake at $\dot{V}_{O_2max}$? Specifically, is O_2 uptake by the unit capillary volume invariant? We find that it is not. In allometric variation, $\dot{V}_{O_2max}/V(c)$ varies as $M_b^{-0.2}$, ranging from 12 ml O_2 min^{-1} ml^{-1} in large animals to 42 ml O_2 ml^{-1} min^{-1} in small (500 g) animals, and it may be even higher in mice and shrews (Gehr *et al.* 1981). In adaptive variation, we find that athletic species load approximately twice as much O_2 into their blood per unit time, a rate that appears to be approximately proportional to their higher haematocrit (Weibel *et al.* 1987; Constantinopol *et al.* 1989). The rate of oxygen uptake by the pulmonary capillary unit is therefore clearly not invariant, in partial contrast to the situation in the muscle capillaries where we have found the discharge rate $\dot{V}_{O_2max}/V(c)$ to be invariant with allometric variation, whereas a similar difference was found between the adaptive pairs. It is noteworthy that the discharge rates are similar in lung and muscle capillaries, at least in the non-athletic species; the observed differences can be explained by different transit times.

When we now consider the total pulmonary diffusing capacity, we must note that it is composed of two main components (a) the membrane diffusing capacity, D_{MO_2}, which is exclusively determined by structural variables, and (b) the blood or erythrocyte diffusing capacity, D_{eO_2}, which depends on capillary blood volume and haematocrit, two parameters that are also subject to some functional variation.

The hypothesis of symmorphosis predicts that D_{MO_2} and D_{LO_2} should be proportional to $\dot{V}_{O_2max}$. This is not what we find. We note that mass-specific D_{LO_2} does not change with size, so that the ratio $D_{LO_2}/\dot{V}_{O_2max}$ increases as $M_b^{0.2}$. This means that a 300 kg cow has six times as much diffusing capacity available as a 30 g mouse to accomplish O_2 uptake at $\dot{V}_{O_2max}$. Therefore, the driving force for O_2 uptake in the lung is smaller in the cow than in the mouse. Why this occurs is unknown. Various possibilities have been suggested, such as (a) appreciable differences in capillary transit time (Lindstedt, 1984) or (b) differences in the pressure head P_{AO_2} as a result of the fact that the size of acini varies considerably with body size (Rodriguez *et al.* 1987; Haefeli-Bleuer and Weibel, 1988), and this could influence alveolar ventilation (Karas *et al.* 1987*a*; Weibel *et al.* 1981*b*).

In adaptive variation, we find that the athletic species have a larger D_{LO_2}/M_b than the non-athletic animals, but this increase is not proportional to the differences in $\dot{V}_{O_2max}$. Here again, athletic species accomplish their higher rate of O_2 uptake by adding to their increased D_{LO_2} an elevated driving force. In this instance, we were able to show that this is partly due to the fact that the athletic species use a greater fraction, approximately 80 %, of their shorter transit time to accomplish equilibration of the capillary blood with alveolar air, whereas the sedentary species use only 50 %, with the remainder appearing as a redundancy (Constantinopol *et al.* 1989; Karas *et al.* 1987*a*).

In conclusion, we find that the ratio $D_{LO_2}/\dot{V}_{O_2max}$ is not invariant, either in allometric or in adaptive variation. To find an invariant ratio, we must consider all structural and functional variables, because only the following relationship applies: $D_{LO_2}/(\dot{V}_{O_2max}/\Delta P_{O_2})$ is invariant.

We must therefore conclude that functional variables are used to a large extent to modulate the rate of O_2 uptake even at $\dot{V}_{O_2max}$. This is possible because the pulmonary gas exchanger maintains an appreciable level of redundancy or excess capacity (Karas *et al.* 1987*a*). One is tempted to speculate that maintaining such redundancy in the part of the respiratory system that forms the interface with the environment may well be a survival strategy allowing the organism to cope with adverse environmental factors such as hypoxia. It has indeed been shown that goats can maintain their $\dot{V}_{O_2max}$ even in high-altitude conditions (Karas *et al.* 1987*a*), presumably because they increase their cardiac output during hypoxia and their gas exchanger is redundant when judged at sea-level conditions.

Conclusions

The limits for the aerobic performance capacity have been assessed in a systematic analysis of the pathway for oxygen from the lung to skeletal muscle mitochondria, taking into account the role played by substrate availability for oxidative metabolism of muscle cells. Current thinking indicates that the 'limitation' is distributed over all levels of the respiratory system, with some steps having more 'resistance' than others under certain conditions. To unravel the basic design principles, we have taken a comparative approach by studying

the structure and function of the respiratory system in animals differing widely in mass-specific $\dot{V}_{O_2max}$. A detailed analysis of each transfer step of oxygen made use of the concept of 'symmorphosis', i.e. assuming, for each level of the respiratory cascade, that animals maintain just enough structure to support flux rates at $\dot{V}_{O_2max}$, but not more. We found all levels of the cascade to conform with the principle of symmorphosis except for the lungs, which seem to be built with significant, though limited, excess structural capacity. However, this redundancy is variable: it is smaller in athletic than in sedentary species, with probably no structural redundancy in the very 'best' of animal (and human) endurance athletes. The analysis also shows that this system is built on the constraint of supplying oxygen rather than substrates to active muscle mitochondria under conditions of maximal aerobic work. Carbohydrate and lipid supply rates are probably 'throttled' by transport processes at the level of the sarcolemma. To ensure adequate substrate supply at high work loads, both lipids and carbohydrates are stored within muscle cells. These substrate stores are replenished at low flux rates during periods of rest to reach a size adequate for high rates of combustion during exercise.

References

BOHR, C. (1909). Über die spezifische Tätigkeit der Lungen bei der respiratorischen Gasaufnahme und ihr Verhalten zu der durch die Alveolarwand stattfindenden Gasdiffusion. *Scand. Arch. Physiol*. **22**, 221–280.

CONLEY, K. E., KAYAR, S. R., RÖSLER, K., HOPPELER, H., WEIBEL, E. R. AND TAYLOR, C. R. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand. IV. Capillaries and their relationship to oxidative capacity. *Respir. Physiol*. **69**, 47–64.

CONSTANTINOPOL, M., JONES, J. H., WEIBEL, E. R., TAYLOR, C. R., LINDHOLM, A. AND KARAS, R. H. (1989). Oxygen transport during exercise in large mammals. II. Oxygen uptake by the pulmonary gas exchanger. *J. appl. Physiol.* **67**, 871–878.

DIPRAMPERO, P. E. (1985). Metabolic and circulatory limitations to $\dot{V}_{O_2max}$ at the whole animal level. *J. exp. Biol.* **115**, 319–332.

GEHR, P., MWANGI, D. K., AMMAN, A., MALOIY, G. M. O., TAYLOR, R. C. AND WEIBEL, E. R. (1981). Design of the mammalian respiratory system. V. Scaling morphometric pulmonary diffusing capacity to body mass: Wild and domestic animals. *Respir. Physiol*. **44**, 61–86.

GOLLNICK, P. D. AND SALTIN, B. (1982). Significance of skeletal oxidative enzyme enhancement with endurance training. *Clin. Physiol.* **2**, 1–12.

HAEFELI-BLEUER, B. AND WEIBEL, E. (1988). Morphometry of the human pulmonary acinus. *Anat. Rec.* **220**, 401–414.

HOLLOSZY, J. O. AND BOOTH, F. W. (1976). Biochemical adaptation to endurance exercise in muscle. *A. Rev. Physiol.* **38**, 273–291.

HOPPELER, H., JONES, J. H., LINDSTEDT, S. L., CLAASSEN, H., LONGWORTH, K. E., TAYLOR, C. R., STRAUB, R. AND LINDHOLM, A. (1987*a*). Relating maximal oxygen consumption to skeletal muscle mitochondria in horses. In *Equine Exercise Physiology*, vol. 2 (ed. J. R. Gillespie and N. E. Robinson), pp. 278–289. Ann Arbor: ICEEP Publications, Edwards Brothers.

HOPPELER, H., KAYAR, S. R., CLAASSEN, H., UHLMANN, E. AND KARAS, R. H. (1987*b*). Adaptive variation in the mammalian respiratory system in relation to energetic demand. III. Skeletal muscles: Setting the demand for oxygen. *Respir. Physiol.* **69**, 27–46.

HOPPELER, H., LINDSTEDT, S. L., UHLMANN, E., NIESEL, A., CRUZ-ORIVE, L. AND WEIBEL, E. R. (1984). Oxygen consumption and the composition of skeletal muscle tissue after training and inactivation in the European woodmouse (*Apodemus sylvaticus*). *J. comp. Physiol.* B **155**, 51–61.

HOPPELER, H., LÜTHI, P., CLAASSEN, H., WEIBEL, E. R. AND HOWALD, H. (1973). The ultrastructure of the normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. *Pflügers Arch.* **344**, 217–232.

HOPPELER, H., MATHIEU, O., KRAUER, R., CLAASSEN, H., ARMSTRONG, R. B. AND WEIBEL, E. R. (1981). Design of the mammalian respiratory system. VI. Distribution of mitochondria and capillaries in various muscles. *Respir. Physiol.* **44**, 87–111.

JONES, J. H., LONGWORTH, K. E., LINDHOLM, A., CONLEY, K. E., KARAS, R. H., KAYAR, S. K. AND TAYLOR, C. R. (1989). Oxygen transport during exercise in large mammals. I. Adaptive variation in oxygen demand. *J. appl. Physiol.* **67**, 862–870.

KARAS, R. H., TAYLOR, C. R., JONES, J. H., LINDSTEDT, S. L., REEVES, R. B. AND WEIBEL, E. R. (1987*a*). Adaptive variation in the mammalian respiratory system in relation to energetic demand. VII. Flow of oxygen across the pulmonary gas exchanger. *Respir. Physiol.* **69**, 101–115.

KARAS, R. H., TAYLOR, C. R., RÖSLER, K. AND HOPPELER, H. (1987*b*). 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.

KLEIBER, M. (1961). *The Fire of Life: an introduction to animal energetics.* Pp. 454. New York, Wiley.

LINDSTEDT, S. L. (1984). Pulmonary transit time and diffusing capacity in mammals. *Am. J. Physiol.* **246**, R384–R388.

LINDSTEDT, S. L. AND CALDER III, W. A. (1981). Body size, physiological time and longevity of homeothermic animals. *Q. Rev. Biol.* **56**, 1–16.

LINDSTEDT, S. L., HOKANSON, J. F., WELLS, D. J., SWAIN, S. D., HOPPELER, H. AND NAVARRO, V. (1991). Running energetics in the pronghorn antelope. *Nature* **353**, 748–750.

MARGULIS, L. (1981). *Symbiosis in Cell Evolution*. San Francisco: Freeman.

MATHIEU, O., KRAUER, R., HOPPELER, H., GEHR, P., LINDSTEDT, S. L., ALEXANDER, R. MCN., TAYLOR, C. R. AND WEIBEL, E. R. (1981). Design of the mammalian respiratory system. VII. Scaling mitochondrial volume in skeletal muscle to body mass. *Respir. Physiol.* **44**, 113–128.

MITCHELL, J. H. AND BLOMQVIST, G. (1971). Maximal oxygen uptake. *N. Engl. J. Med.* **284**, 1018–1022.

PROTHERO, J. (1979). Heart weight as a function of body weight in mammals. *Growth* **43**, 139–150.

PUNTSCHART, A., CLAASSEN, H., JOSTARNDT, K., HOPPELER, H. AND BILLETER, R. (1995). mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance trained athletes. *Am. J. Physiol.* **269**, C619–C625.

RODRIGUEZ, M., BUR, S., FAVRE, A. AND WEIBEL, E. R. (1987). The pulmonary acinus: Geometry and morphometry of the peripheral airway system in rat and rabbit. *Am. J. Anat.* **180**, 143–155.

ROMIJN, J. A., COYLE, E. F., SIDOSSIS, L. S., GASTALDELLI, A., HOROWITZ, J. F., ENDERT, E. AND WOLFE, R. R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* **265**, E380–E391.

SCHMIDT-NIELSEN, K. (1984). *Scaling: Why is Animal Size so Important?* Cambridge: Cambridge Univesity Press.

SUAREZ, R. K. (1992). Hummingbird flight: sustaining the highest mass-specific metabolic rates among vertebrates. *Experientia* **48**, 565–570.

TAYLOR, C. R., KARAS, R. H., WEIBEL, E. R. AND HOPPELER, H. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand. II. Reaching the limits to oxygen flow. *Respir. Physiol.* **69**, 7–26.

TAYLOR, C. R., MALOIY, G. M. O., WEIBEL, E. R., LANGMAN, V. A., KAMAU, J. M. Z., SEEHERMAN, H. J. AND HEGLUND, N. C. (1981). Design of the mammalian respiratory system. III. Scaling maximum aerobic capacity to body mass: wild and domestic mammals. *Respir. Physiol.* **44**, 25–37.

TAYLOR, C. R. AND WEIBEL, E. R. (1981). Design of the mammalian respiratory system. I. Problem and strategy. *Respir. Physiol.* **44**, 1–10.

TAYLOR, C. R., WEIBEL, E. R., HOPPELER, H. AND KARAS, R. H. (1989). Matching structure and function in the respiratory system: Allometric and adaptive variations in energy demand. In *Comparative Pulmonary Physiology: Current Concepts*, chapter 3 (ed. S. C. Wood), pp. 27–65. New York: Marcel Dekker, Inc.

VOCK, R., HOPPELER, H., CLAASSEN, H., WU, D. X. Y., BILLETER, R., WEBER, J. M., TAYLOR, C. R. AND WEIBEL, E. R. (1996*a*). Design of the oxygen and substrate pathways. VI. Structural basis of intracellular substrate supply to mitochondria in muscle cells. *J. exp. Biol.* **199**, 1689–1697.

VOCK, R., WEIBEL, E. R., HOPPELER, H., ORDWAY, G., WEBER, J.-M. AND TAYLOR, C. R. (1996*b*). Design of the oxygen and substrate pathways. V. Structural basis of vascular substrate supply to muscle cells. *J. exp. Biol.* **199**, 1675–1688.

WAGNER, P. D., HOPPELER, H. AND SALTIN, B. (1997). Determinants of maximal oxygen uptake. In *The Lung, Scientific Foundations*, second edition, vol. 2, chapter 153 (ed. R. G. Crystal, J. B. West, E. R. Weibel and P. J. Barnes), pp. 2033–2041. Philadelphia: Lippincott-Raven Publishers.

WEBER, J.-M., BRICHON, G., ZWINGELSTEIN, G., MCCLELLAND, G., SAUCEDO, C., WEIBEL, E. R. AND TAYLOR, C. R. (1996*a*). Design of the oxygen and substrate pathways. IV. Partitioning energy provision from fatty acids. *J. exp. Biol.* **199**, 1667–1674.

WEBER, J. M., ROBERTS, T. J., VOCK, R., WEIBEL, E. R. AND TAYLOR, C. R. (1996*b*). Design of the oxygen and substrate pathways. III. Partitioning energy provision from carbohydrates. *J. exp. Biol.* **199**, 1659–1666.

WEIBEL, E. R. (1997). Design and morphometry of the pulmonary gas exchanger. In *The Lung, Scientific Foundations*, second edition, vol. 1, chapter 82 (ed. R. G. Crystal, J. B. West, E. R. Weibel and P. J. Barnes), pp. 1147–1157. Philadelphia: Lippincott-Raven Publishers.

WEIBEL, E. R., GEHR, P., CRUZ-ORIVE, L. M., MÜLLER, A. E., MWANGI, D. K. AND HAUSSENER, V. (1981*a*). Design of the mammalian respiratory system. IV. Morphometric estimation of pulmonary diffusing capacity, critical evaluation of a new sampling method. *Respir. Physiol.* **44**, 39–59.

WEIBEL, E. R., MARQUES, L. B., CONSTANTINOPOL, M., DOFFEY, F., GEHR, P. AND TAYLOR, C. R. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand. VI. The pulmonary gas exchanger. *Respir. Physiol.* **69**, 81–100.

WEIBEL, E. R., TAYLOR, C. R., GEHR, P., HOPPELER, H., MATHIEU, O. AND MALOIY, G. M. O. (1981*b*). Design of the mammalian respiratory system. IX. Functional and structural limits for oxygen flow. *Respir. Physiol.* **44**, 151–164.

WEIBEL, E. R., TAYLOR, C. R. AND HOPPELER, H. (1991). The concept of symmorphosis: A testable hypothesis of structure – function relationship. *Proc. natn. Acad. Sci. U.S.A.* **88**, 10357–10361.

WEIBEL, E. R., TAYLOR, C. R. AND HOPPELER, H. (1992). Variations in function and design: Testing symmorphosis in the respiratory system. *Respir. Physiol.* **87**, 325–348.