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DESIGN OF THE OXYGEN AND SUBSTRATE PATHWAYS

I. MODEL AND STRATEGY TO TEST SYMMORPHOSIS IN A NETWORK STRUCTURE

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Summary

This first paper in a series develops a model of structure–function relationships for the oxygen and substrate pathways of oxidative metabolism in working muscle. This will be used in the subsequent experimental papers in asking how biological structures are designed if they serve more than one function and whether one function can be served by more than one structural pathway. We have used the concept of *symmorphosis* to address this question; in its original form, it postulates that no more structure is built and maintained at each step in a pathway than is required to meet functional demands. The concept of symmorphosis was developed to deal with the problem of modelling the pathway for oxygen from the environment to mitochondria, essentially a single series of interconnected transfer steps. In the present context, the application of this concept is more complex. Both oxygen and substrates are transported directly from the blood to the mitochondria in what appear to be shared steps. The flows along this direct pathway are adjusted during muscular work. However, substrates have an additional option. They can be stored intracellularly as lipid droplets or glycogen, and thus their supply to mitochondria can occur in two steps separated in time: from capillaries to stores during rest, and from stores to mitochondria during work. The integrated pathways have a network structure and the functional flows are partitioned to different branches of the network, and we must ask whether the partitioning of fluxes is related to design constraints. The principle of symmorphosis predicts that the best use is made of the available options and that the design of each step is matched to the specific functional demand in view of a balance to be achieved over the entire network. This will be tested in subsequent papers by determining maximal flows for oxygen, carbohydrates and lipids through each of the transport steps and their respective structural capacities, comparing dogs and goats, animals of the same size whose maximal oxidative capacities differ by more than twofold. Finally, we will ask whether the principle of symmorphosis can be extended to apply to network systems.

Key words: oxygen consumption, fat oxidation, carbohydrate oxidation, circulation, capillaries, muscle, mitochondria, symmorphosis.

Introduction

The question: economic design of oxidative pathways?

This study addresses a fundamental question of animal design: how are structural systems designed if they serve more than one function? In previous studies, we have argued that animals should not be wasteful in building and maintaining structures that they do not use. We formulated the concept of *symmorphosis*, which postulates that no more structure is built and maintained than is required to meet functional demand (Taylor and Weibel, 1981). We tested this hypothesis on the pathway for oxygen from environmental air to mitochondria. Although we considered a single function, the structure–function relationship did not always turn out to be simple because adaptations to differences in functional demand often involved more than one structure; e.g. the heart, the blood vessels and the red blood cells all contribute to the adaptation to differences in functional demand for oxygen transport. In studies involving many species with up to 10-fold differences in mass-specific aerobic capacity, we found that differences in

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maximal rates of oxygen consumption were matched by equivalent differences in structural capacity at each step in the oxygen pathway, except for the lung. We concluded that these structures are quantitatively adjusted to the maximal rate of O_2 transport and utilization, and that symmorphosis applies to these steps of the oxygen pathway (Taylor *et al.* 1987*b*).

This series of papers extends our previous studies to include, besides O_2, the supply of substrates, glucose and fatty acids, to the mitochondria. Generally speaking, the oxygen and substrate pathways converge in the mitochondria; they take partly different routes, but there are several steps along the way where the same structures appear to transport both. Furthermore, whereas during work O_2 must be supplied directly from ambient air to the mitochondria, substrates are, in part, immediately available inside the working muscle cells in the form of intracellular lipid droplets and glycogen deposits, which are stocked-piled during periods of rest. The substrate pathways are therefore not linear but rather show a network structure.

The general question we ask in this series of papers is how the network design of these pathways is quantitatively adjusted to serve the delivery of both O_2 and substrates efficiently, making best use of the different options available, and whether the structures supporting these pathways are matched to the functional needs. Finally, we will ask whether the concept of symmorphosis is useful in an analysis of partitioned functions in relation to network structures.

Network model for matched O_2 and substrate supply in overview

We develop a model of the O_2 and substrate pathways which defines the relevant structural and functional variables. The purpose of this model is to establish the quantitative relationships between the fluxes of oxygen and substrates and the structures that support these fluxes. We include only carbohydrates and lipids, ignoring proteins because their energy contribution is minimal during exercise (Rennie *et al.* 1981; Carraro *et al.* 1994). The model in Fig. 1 shows a muscle cell with a mitochondrion (the end-point of the pathways) at the bottom connected by the circulation to the sources of O_2 and substrates at the top. The three pathways are indicated with different symbols: dots for O_2, circles for fatty acids and squares for glucose. Arrows denote where transport processes occur.

Oxygen – a single pathway

Oxygen follows a single pathway without branches, and the flux rates through all of the steps are equal. This is because oxygen stores in the myoglobin are small and steady-state O_2 flux rates are reached after a few minutes. Then, all the oxygen consumed in the mitochondria is supplied *via* the lung and blood from environmental air. The highest rates of O_2 consumption in the muscle mitochondria coincide with the highest rates of O_2 transport by the circulation and in the lung.

Substrates – branched pathways

The substrate pathways are complex: carbohydrate and lipid pathways are parallel and both branch, forming four pathways which converge at the site of oxidation. More importantly, the maximal rate of substrate uptake from the gut does not occur at the same time as the maximal oxidation rate in the muscle cells. It occurs during rest, when oxidation rate is near its minimum. In turn, maximum oxidation rate occurs during exercise, when substrate uptake is at its minimum. Thus, uptake and oxidation are separated in time and regulated independently. Stores serve as buffers to offset this temporal shift.

Substrate uptake

Maximum rates of substrate uptake from the gut to the circulation (Fig. 1) occur after meals, when the body is in an absorptive state. Lipid and carbohydrate uptake in the gut take different routes. Lipids are taken up by the brush-border membrane and resynthesized to triglycerides or phospholipids in intestinal epithelial cells. Their transfer to the blood (in the form of lipoproteins and chylomicrons) occurs mostly through the intestinal lymphatic system. Carbohydrates are taken up across the intestinal epithelium into the interstitial space. Two parallel, interdependent pathways are involved: a transcellular pathway driven by active transport, and a paracellular pathway where dissolved substrates are transported passively between the cells by solvent drag (Pappenheimer, 1993). These pathways have a ‘safety margin’ of about two, and the structures are extremely malleable. Diamond and his colleagues have found that the structures involved increase by two- to threefold in response to the increased demands induced by lactation or cold (Diamond and Hammond, 1992).

The transfer from gut to stores is *via* the circulation (Fig. 1). Glucose is very soluble and is simply dissolved in the plasma for transport. Fatty acids and triglycerides, however, are very insoluble and are transported in the form of chylomicrons (found in high concentrations in the blood a few hours after meals) as well as bound to albumin. It is interesting that the structural capacity of albumin for binding fatty acids is adjusted to differences in maximal energetic demand, i.e. dog albumin binds 1.5 times as much as goat albumin (McClelland *et al.* 1994). During absorption, most of the blood flow is directed to the digestive organs and stores, with only about 10–20 % going to the skeletal muscle (Folkow and Neil, 1971; Choshniak *et al.* 1995). Substrates flow mainly to the stores (Fig. 1): the liver (which stores both carbohydrates and lipids), the adipose tissue (which stores only lipids) and intracellular stores in the muscle cells (which store both carbohydrates and lipids). These organismic stores are capable of stocking enough energy to fuel oxidation for weeks to months without food.

The importance of stores

During exercise, when rates of substrate oxidation are highest, blood flow is shunted away from the gut to active muscles, and rates of substrate uptake are lowest. Under these

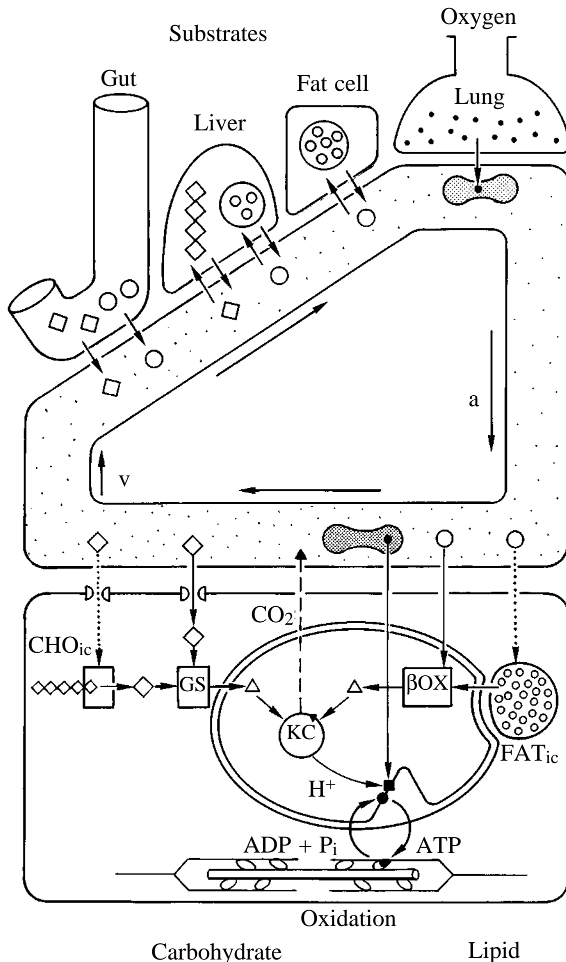

Fig. 1. Network model of the oxygen and substrate pathways from the supply organs (lung, gut, liver, fat cells) through the circulation (a, arterial; v, venous) to the muscle cells (bottom) with mitochondrion, intracellular fat droplet (FAT_{ic}), glycogen granules (CHO_{ic}) and actomyosin complex. Substrates are oxidized at the terminal oxidase in the inner mitochondrial membrane (black square), generating the energy used to phosphorylate, at the associated F_1-ATPase, ADP to ATP. The oxygen pathway is marked with black dots, the fatty acid pathway with circles and the glucose pathway with squares. Arrows indicate transport processes between compartments. Oxygen is supplied to the blood in the lung; substrates are taken up in the gut and liver, and adipose tissue (fat cells) serve as a buffer for glucose and fatty acid concentration in the blood. The glucose uptake by the muscle cell is mediated by GLUT transporters (paired semicircles) in the sarcolemma. Note that the substrate pathways from the microcirculation to the mitochondria are split into a direct and an indirect pathway. The direct pathways (solid arrows) lead directly to the sites of glycolysis (GS) in the cytosol and of β-oxidation (βOX) in the mitochondria. The indirect pathways lead through intracellular stores of glycogen (row of squares, CHO_{ic}) and of lipid droplets (FAT_{ic}); the dotted arrows indicate that substrate supply from the blood to the stores is temporally offset from utilisation. The breakdown product of glycolysis and β-oxidation, acetyl-CoA (triangles), enters the Krebs tricarboxylic acid cycle (KC) to generate reducing equivalents (H^+) for oxidation, releasing CO_2 that diffuses to the capillaries (broken arrow) for discharge through the lung.

conditions, the stores must supply almost all of the fuel for oxidation. Stores function as buffers, allowing substrates to be transferred at different rates in different steps of their pathways, even under conditions of maximal oxidation. In contrast, the oxygen pathway lacks significant stores. Oxygen is always available from the environmental air and can be transferred quickly because it involves simple diffusion of a small molecule (soluble in both lipids and water) and convective transport in the blood at high concentrations.

Substrate oxidation

Substrate flows to muscle mitochondria reach their maximal rates during exercise. As exercise intensity increases from rest to maximal rates of oxidation, blood flow to the active muscles increases by 20- to 30-fold, until about 90 % of the blood flow is directed to the skeletal muscles (Armstrong *et al.* 1992). These high flows are necessary to deliver the oxygen, and possibly the substrates for oxidation, and to carry away the carbon dioxide and heat that are produced. Glucose and fatty acids are mobilized from the liver and adipose tissue and transported by the circulation to the muscle cells (Fig. 1). Muscle cells also draw on their intracellular stores of substrates. The intracellular pathways from glycogen and lipid droplets to the sites of oxidation are short and uncomplicated by interposed structures. The transfer of substrates from these stores to the sites of catabolism, glycolysis or β-oxidation, is regulated by the energetic demand of the cells as well as by hormones and the autonomic nervous system.

A quantitative model

The pathways

What are the relevant morphological parameters that could limit maximal flux rates of oxygen and substrates? The maximal rate of oxidation in the mitochondria ($\dot{M}_{O_2max}$) is proportional to mitochondrial volume (Hoppeler and Lindstedt, 1985; Hoppeler *et al.* 1987). On a finer scale, $\dot{M}_{O_2max}$ is proportional to the inner mitochondrial membrane surface area, S(im), where the respiratory chain enzymes are located. It is here that oxygen is consumed to phosphorylate ADP. Substrates, in contrast, are broken down in the Krebs cycle (KC, see Fig. 1) to generate reducing equivalents. This takes place in the mitochondrial matrix. Thus, matrix volume [V(ma), the volume within mitochondria exclusive of the inner membranes] is an appropriate structural parameter for substrate catabolism, assuming a constant density of enzymes in the matrix space. Matrix volume appears to be proportional to mitochondrial volume for the dog and goat as well as for most mammals.

The maximal rate of substrate oxidation is equal to the maximal rate of oxygen consumption. However, the contributions from carbohydrates and fatty acids must be considered separately. The relative contributions of the two substrates can be estimated by indirect calorimetry. This allows partitioning of the mitochondrial O_2 consumption rate into the

oxidation rate of carbohydrates, $\dot{M}^{\mathrm{CHO}}_{\mathrm{O_2}}(\mathrm{mt})$, and that of free fatty acids, $\dot{M}^{\mathrm{FFA}}_{\mathrm{O_2}}(\mathrm{mt})$:

$$\dot{M}_{\mathrm{O_2}}(\mathrm{mt}) = \dot{M}^{\mathrm{CHO}}_{\mathrm{O_2}}(\mathrm{mt}) + \dot{M}^{\mathrm{FFA}}_{\mathrm{O_2}}(\mathrm{mt})\,, \qquad (1)$$

where all the rates are expressed in oxygen equivalents.

Equation 1 can also be expressed in molar equivalents of oxygen and substrates, since the oxidation of 1 mol of glucose consumes 6 mol of O_2, and the oxidation of 1 mol of fatty acids consumes 23 mol of O_2:

$$\dot{M}_{\mathrm{O_2}}(\mathrm{mt}) = 6\dot{M}_{\mathrm{CHO}}(\mathrm{mt}) + 23\dot{M}_{\mathrm{FFA}}(\mathrm{mt})\,. \qquad (2)$$

Both substrates provide acetyl-CoA to the Krebs cycle (Fig. 1). Fatty acids produce acetyl-CoA by β-oxidation in the mitochondrial matrix, while glucose produces it by a two-step process, beginning with the formation of pyruvate (glycolysis) in the cytosol. The pyruvate is then shuttled into the mitochondrial matrix where the dehydrogenase complex converts it to acetyl-CoA. The distances involved in transport and the surface areas of mitochondrial membranes, glycogen granules and lipid droplets are important structural parameters that we incorporate in our analysis. We have not attempted to localize the enzymes involved in these processes at this time; however, these measurements seem feasible and could be undertaken in the future.

The carbohydrate and fatty acid pathways are separate in the cytosol. Each substrate has two pathways to the mitochondria: one from intracellular stores (ic) and one from intravascular pools (iv), so:

$$\dot{M}_{\mathrm{CHO}}(\mathrm{mt}) = \dot{M}_{\mathrm{CHO}}(\mathrm{ic}) + \dot{M}_{\mathrm{CHO}}(\mathrm{iv})\,, \qquad (3)$$

$$\dot{M}_{\mathrm{FFA}}(\mathrm{mt}) = \dot{M}_{\mathrm{FFA}}(\mathrm{ic}) + \dot{M}_{\mathrm{FFA}}(\mathrm{iv})\,. \qquad (4)$$

The pathways from the intracellular pools are short for carbohydrates and approach zero for fats (see paper VI; Vock *et al.* 1996*a*). Thus, these stores provide the mitochondria with almost immediate access to substrates. The pathways from the plasma pools, however, must traverse several barriers – capillary endothelium, interstitial space and sarcolemma – before they reach the cytosol (Fig. 2). Flux rates from intravascular pools are limited by the design properties of these structures, and we will use the difference in maximal rates between dogs and goats, together with a quantitative analysis of these structures, to calculate the extent of this limitation (Vock *et al.* 1996*b*).

Carbohydrate supply

Let us first consider carbohydrates. The maximum rate of carbohydrate oxidation depends on the rate at which glycolysis (GS in Fig. 1) can produce acetyl-CoA from glycogen stores in the cell and from glucose supplied by the circulation (equation 3). The structural correlates for $\dot{M}_{\mathrm{CHO}}(\mathrm{ic})$ are not easily quantified. Carbohydrate is stored in glycogen granules which are dispersed throughout the sarcoplasm (Fig. 3). Some are aggregated in packets that may be closely associated with mitochondria, but others are distant from any mitochondria and may be involved only in anaerobic glycolysis. We have used the concentration of glycogen in the muscle cells, $C_{\mathrm{gluc}}(\mathrm{f})$, as a design parameter for carbohydrate store size. Concentration can be expressed in moles of polymerized glucose per volume of muscle fibre and can be estimated biochemically. Since glycogen granules in muscle are fairly uniform in size and shape, this concentration is also a relative measure of the total surface area of the granules from which glucose is liberated by glycogenolysis. The granules supply anaerobic glycolysis as well as the oxidative pathway. Since glycogen can be

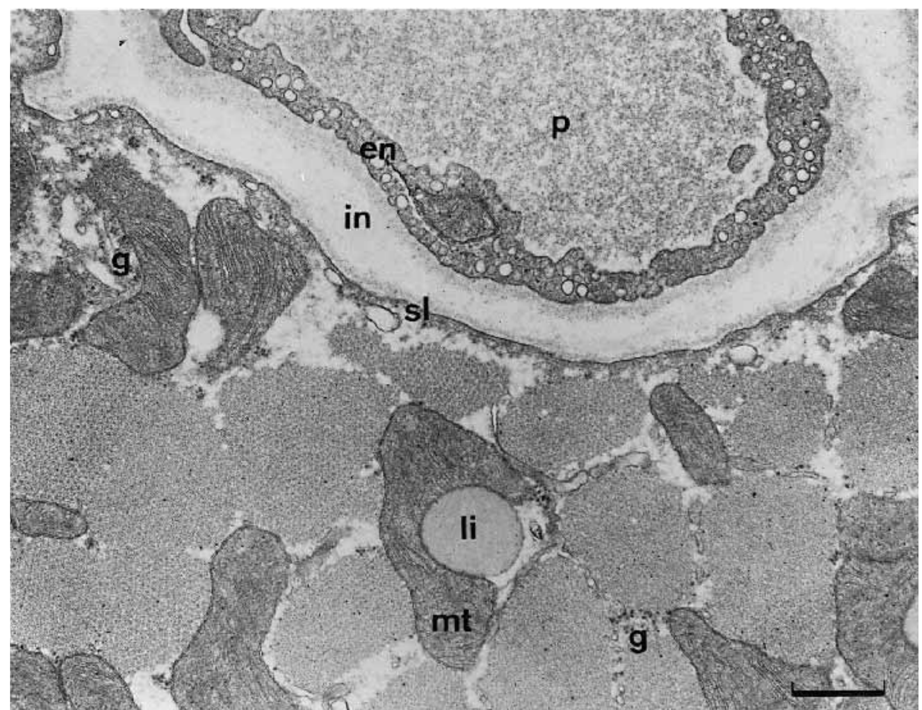

Fig. 2. Transmission electron micrograph of dog triceps muscle showing the pathway that substrates must follow from the plasma (p) through the endothelium (en), the interstitium (in) and the sarcolemma (sl) into a skeletal muscle cell. Here, the substrates are either immediately oxidized in the mitochondria (mt) or stored in the form of glycogen granules (g) and lipid droplets (li). Scale bar, 0.5 μm.

mobilized almost 200 times as rapidly for anaerobic glycolysis as for oxidation (Margaria, 1976), the surface area of the granules may be related more to the rate of glycolysis than to maximal rates of oxidation.

The pathway supplying glucose from the circulation, $\dot{M}_{CHO}(iv)$, traverses the capillary endothelium, the interstitial space, the sarcolemma and the myocytoplasm to reach the sites of glycolysis. These sites are dispersed throughout the sarcoplasm, because glycolysis provides ATP directly to the myofibrils, as well as the pyruvate that is shuttled into the mitochondria. The flux rate of glucose from the plasma pool into the muscle cell depends on the glucose concentration difference between plasma (p) and cytosol (cy), and on an overall conductance G_{gluc}:

$$\dot{M}_{CHO}(iv) = [C_{gluc}(p) - C_{gluc}(cy)]G_{gluc}\,. \qquad (5)$$

The glucose concentration in the plasma can easily be measured (Weber *et al.* 1996*b*), but the concentration of free glucose in the working muscle cell is unknown. The overall conductance G_{gluc} can be broken into four serial conductances for endothelium (en), interstitium (in), sarcolemma (sl) and cytosol (cy):

$$G_{gluc}^{-1} = G_{gluc}(en)^{-1} + G_{gluc}(in)^{-1} + G_{gluc}(sl)^{-1} + G_{gluc}(cy)^{-1}\,. \qquad (6)$$

Since glucose is transported by diffusion (facilitated in the sarcolemma), these conductances take the general form:

$$G_{gluc}(x) = D_{gluc}(x)S(x)/l(x)\,, \qquad (7)$$

where $D_{gluc}(x)$ is a transfer coefficient, $S(x)$ is the effective diffusion surface area and $l(x)$ is the diffusion distance. We do not yet possess an adequate model to describe all of these conductances in terms of specific transfer coefficients and design parameters. However, the most significant design parameters are the surface areas of the membranes, i.e. capillary surface area, $S(c)$, and sarcolemmal surface area, $S(sl)$, which can be measured (Vock *et al.* 1996*b*). The distances to be traversed (Fig. 2) depend on the proximity of these surfaces; for example, $l(in)$ will depend on the relative densities of capillary and sarcolemmal surface in the tissue volume and on the volume density of the interstitial space. The hydrophilic glucose molecule can easily diffuse through the ‘leaky’ endothelial junctions of the capillaries (Pappenheimer, 1953) and the aqueous spaces of the interstitium and cytosol. However, transporters are required for glucose to cross the sarcolemma (Mueckler, 1994). The conductance of the endothelium will depend primarily on the length density of junctions (see Vock *et al.* 1996*b*, for a description and discussion of this parameter), while the conductance of the sarcolemma will depend primarily on the density of glucose transporters (GLUT-1 and GLUT-4) (Goodyear *et al.* 1991; Mueckler, 1994).

Fatty acid supply

Let us now consider the transport of fatty acids from the intracellular stores, $\dot{M}_{FFA}(ic)$, and the circulation, $\dot{M}_{FFA}(iv)$, to the sites of β-oxidation in the matrix of the mitochondria (βox in Fig. 1). The maximum rate of fatty acid oxidation will depend on the rate at which β-oxidation can produce acetyl-CoA from these two sources (equation 4). The structural correlates for $\dot{M}_{FFA}(ic)$ are the relative volume of intracellular lipid droplets (li) in the muscle fibre (f), termed $V_V(li,f)$, and the contact surface area between the lipid droplets and the mitochondrial outer membranes (om), since we have observed that all the lipid droplets in muscle cells are in direct contact

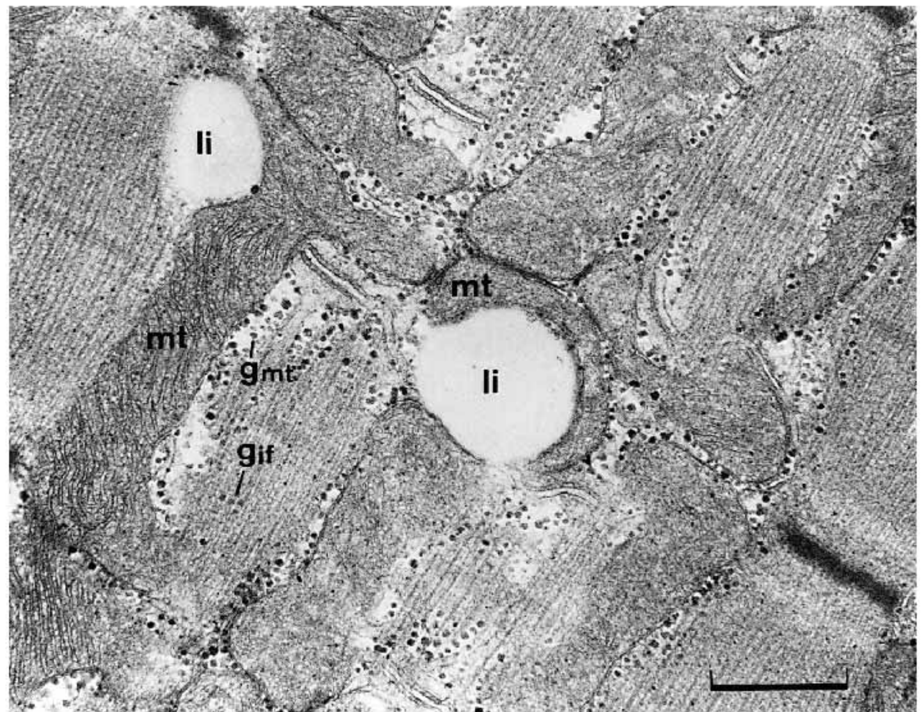

Fig. 3. Transmission electron micrograph of dog triceps muscle showing intracellular substrate stores such as glycogen granules (g) and lipid droplets (li). Glycogen is found either in packets associated with mitochondria (g_{mt}) or interfibrillarly dispersed (g_{if}); the lipid droplets, however, are always in close contact with mitochondria (mt), exhibiting a distinct contact line from which the contact surface area between lipid stores and outer mitochondrial membranes can be estimated. Scale bar, 0.5 μm.

with the outer mitochondrial membrane (Fig. 3). We propose that the transfer of fatty acids occurs exclusively at these contact sites, which we designate (li-om). Accordingly, the morphometric parameters determining the transfer are the fractions of the lipid and mitochondrial outer membrane surface areas occupied by these contact sites: S_S(li-om, li) and S_S(li-om, om), respectively. The release of fatty acids from the triglycerides stored in the lipid droplets involves a lipase. The close association between lipid droplets and mitochondria suggests that this enzyme may be bound to the outer mitochondrial membrane together with the carnitine shuttle system that moves fatty acids into the mitochondrial matrix. This arrangement would maximize the transfer of fatty acids to the sites of β-oxidation and circumvent transfer problems associated with the low solubility of fatty acids.

The transfer of fatty acids from the intravascular pool can be described by an equation similar to the one for glucose:

$$\dot{M}_{\mathrm{FFA}}(\mathrm{iv}) = [C_{\mathrm{FFA}}(\mathrm{p}) - C_{\mathrm{FFA}}(\mathrm{cy})]G_{\mathrm{FFA}}\,. \tag{8}$$

The overall conductance G_{FFA} can again be broken into four serial conductances. The transfer conditions are, however, quite different from those for glucose because of the low solubility of fatty acids in aqueous media. In the plasma and interstitial spaces, fatty acids are bound to albumin and in the cytosol they are bound to fatty-acid-binding proteins. As a result, the effective concentrations are not easily defined. In contrast, fatty acids can diffuse across the lipid phase of the plasma membranes and it is uncertain whether sarcolemmal fatty acid transporters are involved (Stremmel *et al.* 1992; Higgins, 1994). The morphometric parameters that affect the four conductances are the same as for the glucose pathway: the capillary endothelial surface area, the sarcolemmal surface area and the distances to be traversed across the endothelium, the interstitium and the sarcolemma.

Questions and hypothesis

Our model describes, in addition to the O_2 pathway, the supply of substrates for oxidation in the mitochondria through four different but linked pathways, each with different structures and different functional characteristics. They can be summarized as follows. For fatty acids as well as carbohydrates, the flux from blood to mitochondria can be direct and its rate will therefore be determined largely by the conductances of the endothelium and sarcolemma. Alternatively, these fluxes can occur indirectly in two steps separated in time, the first leading from blood plasma to intracellular stores, lipid droplets and glycogen granules, and the second from these stores to the mitochondria. In the two-step process, the supply of substrates to the cells occurs mainly during periods of rest at comparatively low rates; during exercise, the flux of substrates from the stores to the mitochondria is independent of the flow limitations imposed by the endothelium and sarcolemmal barriers but depends only on the (quite intimate) relationships between the mitochondria and intracellular stores. By virtue of these intracellular stores, the substrate pathways differ fundamentally from that for O_2, for which only the direct route is available. Design properties are shared by the O_2 and substrate pathways in a critical way only along the direct route.

On the basis of these general conditions for O_2 and substrate supply, we ask four questions which we attempt to answer by exploiting the twofold difference in maximal rates of oxidative metabolism between dogs and goats.

(1) How is substrate supply partitioned between the four pathways as the rate of fuel oxidation is increased to maximal rates during exercise, and does this differ between dogs and goats?

(2) Are there differences between the two species in the design properties of each branch and of each step of the pathways that are matched to differences in the flux rates through that branch?

(3) Are there functional limitations in any of these transfer steps that appear to be related to design properties?

(4) Are there parts of this network structure that are designed specifically for O_2 or for substrate supply?

In the context of structural pathways that are shared by different but related functions and furthermore show network characteristics, the test of the general hypothesis of symmorphosis cannot be approached with the simple question: how much structure is enough? The structures need not all be co-adjusted in a simple way. Where parallel transport options exist, we must ask whether the partitioning of fluxes is related to design constraints in the different branches of the network. The principle of symmorphosis then predicts, in the sense of a broad general hypothesis, that the best use is made of the options offered by the design of the system and that the design of each step is matched or adjusted to the specific functional demand in accordance with the overall functional needs.

Experiments to test the model

In this study of substrate pathways, we have decided to limit ourselves to conditions of maximal oxidation rate. Even with this limitation, we are still left with a large task. Measuring the functional variables characterizing O_2 and substrate flux rates requires extensive physiological and biochemical studies. Quantifying the relevant structures demands new approaches to morphometry. In most cases, we were able to carry out both the physiological and the morphometric studies on the same animals, but in a few cases this was not possible.

To tackle this complex task, we use a comparative approach to obtain large differences in fluxes through the entire system. We take advantage of an experiment of nature by comparing dogs and goats, animals of the same size that are adapted for very different levels of aerobic performance: the capacity for aerobic oxidation of the dog is about twice that of the goat (Taylor *et al.* 1987*a*). We have already gathered extensive data on the steps in the oxygen pathway for these species. This allows us to focus on the substrate pathways. But we will have to establish that dogs and goats are comparable for our purposes even though they acquire their substrates from food

in very different ways, as carnivores and ruminants, respectively.

Animals of each species were trained to run on a treadmill at speeds up to and including those eliciting maximal rates of oxidation. At each exercise intensity, the relative rates of carbohydrate and fat oxidation were determined using indirect calorimetry (Roberts *et al.* 1996). This permitted the determination of the exercise intensities at which the maximal rates of fatty acid and carbohydrate oxidation occurred and also served as a test for the comparability of our two experimental species. Then, the rates of oxidation of circulating glucose and fatty acids were determined using infusions of radioactively labelled glucose and palmitate, respectively. Subtracting these rates from the total oxidation rates yielded the rate of oxidation of intracellular stores of glucose and fatty acids (Weber *et al.* 1996*a*,*b*). After the completion of the physiological studies, the animals were killed, and representative samples of the entire skeletal muscle mass were collected for morphometric and biochemical analyses (Vock *et al.* 1996*a*,*b*). In the final paper of this series, we draw general conclusions about the design of the converging pathways of oxygen and substrates for fuelling muscular work and ask whether our findings support or contradict the principle of symmorphosis applied to a network system (Weibel *et al.* 1996).

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