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DESIGN OF THE OXYGEN AND SUBSTRATE PATHWAYS

V. STRUCTURAL BASIS OF VASCULAR SUBSTRATE SUPPLY TO MUSCLE CELLS

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Summary

This paper quantifies the structural capacity of the transport steps for oxygen, glucose and fatty acids from the blood in capillaries to the cytosol of muscle cells and compares it with maximal rates of oxygen and substrate transport measured in the same animals and reported in the preceding papers of this series. Dogs have relatively more muscle per unit body mass than goats (37 *versus* 26 %), but the maximal rate of oxidation per gram of muscle is still larger in the dog by a factor of 1.55. The maximal rates of substrate supply from the circulation are similar in both species. We predict that these differences in physiological parameters should be matched by proportional differences in structural capacity. We find that capillary volume and surface area are matched to maximal oxygen demand. The rate of vascular substrate supply is proportional neither to the capillary surface area nor to the length of intercellular junctions. The sarcolemmal surface area per gram of muscle is the same in both species. Using the physiological data presented in the companion papers of this series, we have calculated the maximal flux densities of circulatory glucose and fatty acids across the capillary wall and the sarcolemma. We find, for both substrates, that the flux densities across the sarcolemma reach a maximum at nearly the same level and at low exercise intensities in both species. In contrast, the flux densities across the capillary surface and the endothelial junctions are higher in goats than in dogs. We conclude that the capillaries are designed for O_2 supply up to maximal rates of oxidation but not for the supply of the substrates (glucose and fatty acids) at the rates required at high exercise intensities. These are limited by the transport capacities of the sarcolemma.

Key words: muscle oxidative metabolism, capillaries, sarcolemma, glucose supply, fatty acid supply, substrate flux densities, symmorphosis, dog, goat.

Introduction

In this series of studies, we ask how structures are designed if they serve more than one function. We address this question by considering specifically the structures of the respiratory system which supply not only O_2 to the mitochondria of muscle cells but also the substrates required for oxidative metabolism: carbohydrates and fatty acids. While the pathways for O_2 and substrates are separated at their central sources (the lung for O_2; the gut and liver for carbohydrates; the gut and adipose tissue for fatty acids), all three use the blood and circulation for their transport and delivery to the peripheral muscle cells. However, this common pathway is only partly shared as O_2 is carried in erythrocytes and substrates in plasma.

Previous studies comparing animals with different maximum rates of oxygen consumption have shown that the capillary bed and its erythrocytes are quantitatively adjusted to the maximal oxygen needs of the muscles, supporting the hypothesis of symmorphosis (Hoppeler *et al.* 1987, 1991; Conley *et al.* 1987; Weibel *et al.* 1992). However, this result may be fortuitous. Because capillaries supply both O_2 and substrates to the muscle cells, it may be that the microvasculature is designed for substrate supply and that this would provide sufficient capacity for O_2 supply. Here, we therefore expand the concept of symmorphosis previously tested for the linear pathway for O_2 to a network system of branching and converging pathways with partly shared functions. We postulate that the design of each step is adjusted to the specific functional demand necessary to achieve a balanced function of the entire network (Taylor *et al.* 1996). We therefore ask whether the microvascular structures are quantitatively adjusted to the maximal rates of substrate supply or whether we can determine the structural limitations of this system responsible for the partitioning of substrate fluxes.

For this purpose, we compare maximal flux densities across the

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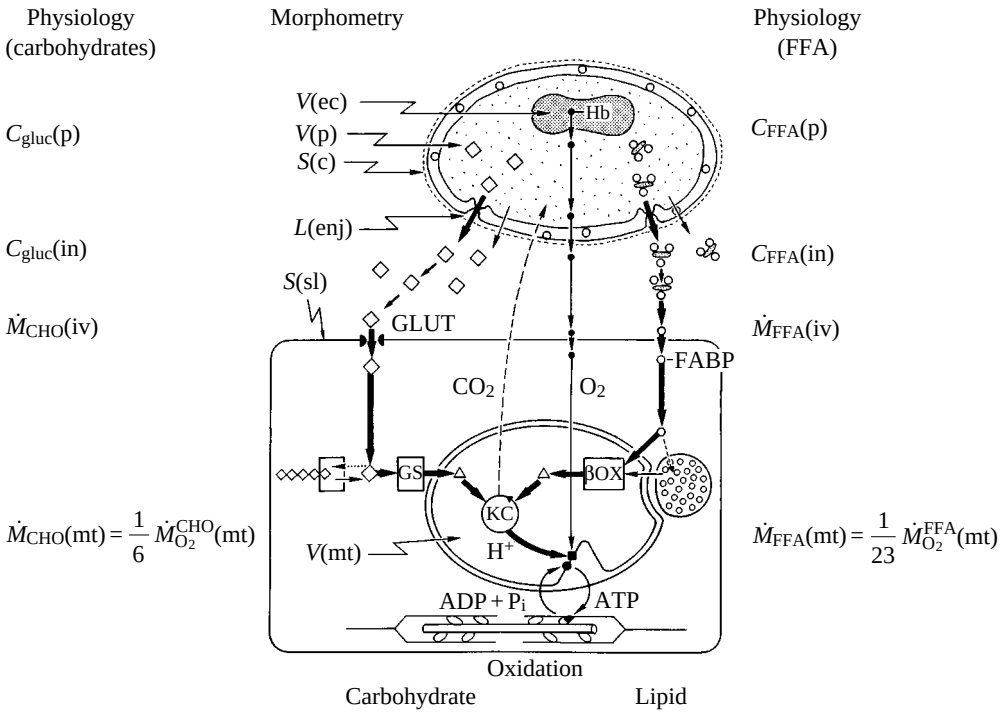

Fig. 1. Model for structure–function relationships of oxygen and substrate supply from the microvasculature to the skeletal muscle cell and its mitochondria. Dots indicate oxygen, open circles fatty acids (bound to albumin in plasma and interstitial space), squares glucose and triangles acetyl-CoA. Heavy arrows mark the transfer pathways for substrates from plasma to the terminal mitochondrial oxidase (black square). The substrate pathways out of the capillary (c), containing plasma (p) and erythrocytes (ec) with haemoglobin (Hb), can traverse the endothelium (thin arrow) or the endothelial junctions (enj, thick arrow). Glucose crosses the sarcolemma (sl) through transporters of the GLUT family, marked by paired hemicircles. Morphometric parameters (*V*, volume; *S*, surface area; *L*, length) are listed together with the correlated physiological parameters: *C*, concentration of glucose (gluc) and fatty acids (FFA) in plasma (p) and interstitium (in); $\dot{M}_{CHO}$(iv) and $\dot{M}_{FFA}$(iv), molar flux rates of carbohydrates (CHO) or fatty acids (FFA) from intravascular pool (iv); $\dot{M}_{CHO}$(mt) and $\dot{M}_{FFA}$(mt), molar rates of carbohydrate and fatty acid consumption in muscle mitochondria (mt), which are proportional to mitochondrial O_2 consumption induced by these two substrates $\dot{M}_{O_2}^{CHO}$(mt) and $\dot{M}_{O_2}^{FFA}$(mt), respectively. GS, glycolysis; βOX, β-oxidation; KC, Krebs cycle; H^+, reducing equivalents; FABP, fatty-acid-binding protein.

capillary and sarcolemmal barriers of the substrate pathway (Fig. 1) in two animals of the same size but with a 2.2-fold difference in maximal rates of oxidation. Flux densities are calculated from morphometric measurements of the structures involved (this study) and maximal rates of carbohydrate and fatty acid fluxes from the circulation to muscle cells measured in the same animals during exercise at different intensities (Roberts *et al.* 1996; Weber *et al.* 1996*a*,*b*). Substrate flux rates from capillaries to myocytes were determined using tritiated water as a tracer for carbohydrate flux and ^{13}C-labelled palmitate as a tracer for fatty acid flux. These substrate flux studies have revealed two limitations in the transport of substrates out of the circulation: (1) maximal flux rates of both glucose and fatty acids are reached at work intensities of approximately 40 % $\dot{V}_{O_2max}$ and do not increase appreciably with higher exercise intensities; and (2) maximal rates of circulatory substrate supply are similar in dogs and goats, i.e. they do not parallel the 2.2-fold difference in maximal rates of oxidation (Weber *et al.* 1996*a*,*b*). Given the similar transcapillary substrate flux rates in both species plateauing at similar (low) work intensities, we hypothesized that this limitation could be imposed by the structural design of the transfer steps, which we therefore expected to be similar in dogs and goats.

Model for structure–function relationships of substrate supply from the circulation

The model of structure–function relationships for O_2 and substrate supply from the capillary to the muscle cell is shown in Fig. 1 (Taylor *et al.* 1996). The functional variables are the flux rates of glucose and fatty acids from the intravascular pools, $\dot{M}_{CHO}$(iv) and $\dot{M}_{FFA}$(iv). These have been measured at exercise intensities of 40, 60 and 85 % $\dot{M}_{O_2max}$ as a fraction of total substrate consumption by the mitochondria, $\dot{M}_{CHO}$(mt) and $\dot{M}_{FFA}$(mt), using appropriate tracer techniques (Weber *et al.* 1996*a*,*b*).

The pathway supporting $\dot{M}_{CHO}$(iv) and $\dot{M}_{FFA}$(iv) begins in the blood plasma. The volume of plasma, *V*(p), together with the concentration of the substrate, quantitatively determine the intravascular stores available. The substrates drawn from the capillary plasma space must traverse several barriers and tissue compartments before they can enter the mitochondria: endothelium, interstitial space, sarcolemma and myocytoplasm (Fig. 2). Glucose can diffuse freely in the aqueous spaces of the plasma, interstitial space and myocytoplasm, but the hydrophobic fatty acids must be solubilized by binding to albumin or to cytoplasmic fatty-acid-binding proteins (FABPs).

The conductances of the barriers between compartments are determined by the surface area of the 'membranes' mediating transfer. In contrast to that for O_2, the transfer of substrates is not governed simply by the laws of diffusion, but may involve specific transporters. The transfer conditions will be different for sarcolemma and endothelium. The sarcolemma acts as a simple cell membrane completely separating the

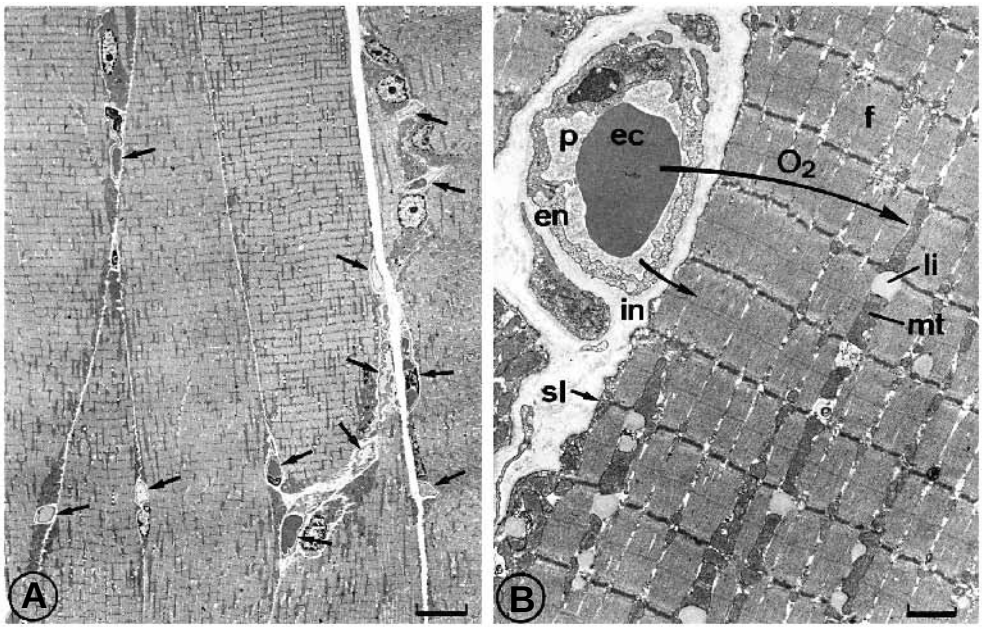

Fig. 2. Transmission electron micrographs from isotropic uniform random (IUR) sections of dog triceps muscle. (A) Low power: capillaries are indicated by arrows. (B) Intermediate power: pathways for oxygen beginning at the erythrocyte (ec) and for substrates beginning in the plasma (p) are indicated as crossing the endothelium (en), the interstitial space (in) and the sarcolemma (sl). The muscle cells contain myofibrils (f), mitochondria (mt) and lipid droplets (li). Scale bars, A, 10 μm; B, 1 μm.

myocytoplasm from the interstitial space. In contrast, the endothelial barrier consists of cytoplasmic leaflets bounded by two plasma membranes. Aqueous channels may exist between plasma and interstitial space at the intercellular junctions which are important for the transfer of substrates, mainly of glucose (Pappenheimer, 1953; Michel, 1988). These structural differences will have specific effects on the transfer of substrates across these barriers.

The capillary endothelium as an exchange barrier

The capillary wall has two domains that differ in terms of their possible permeability characteristics: the endothelial cell, consisting of two cell membranes separated by a layer of cytoplasm, and the intercellular 'tight' junction bands (Fig. 3).

Pappenheimer (1953) developed the idea that, in muscle capillaries, 'specialized regions through or between endothelial cells' endow the capillary wall with a high degree of permeability for non-lipophilic solutes such as glucose. More recent evidence has reinforced this idea as it has repeatedly been shown that capillary endothelial junctions are 'leaky' compared, for example, with epithelial junctions. The junction band is a reticular structure with discontinuities in the fibrillar network that can serve as hydrophilic channels between the plasma and the interstitial space (Perry, 1980; Adamson and Michel, 1993).

The simplest model for the transfer of hydrophilic solutes such as glucose from plasma to interstitial fluid is to consider the pores at the endothelial junctions as a slit-like structure. Their morphometric characteristics are the slit length, l, the slit width, w, and the channel length, Δx. The basic measure of slit length is the length of the endothelial junction, L(enj); Adamson and Michel (1993) estimated in frog mesenteric capillaries that about 3 % of the junction length represents open slits, but it is likely that this value is different, probably smaller, in muscle capillaries. The slit width has been estimated from measurements of permeability to be in the nanometre range; for frog mesentery capillaries, Crone and Levitt (1984) calculate $w \approx 13$ nm, whereas Pappenheimer (1953) estimated $w \approx 4$ nm for muscle capillaries, a value which has been repeatedly confirmed. The channel length is estimated to be less than 1 μm (Crone and Levitt, 1984), but this depends on the geometry of the channel (Perry, 1980). To avoid these uncertainties, Pappenheimer (1953) has introduced the compound parameter 'restricted pore area per unit path length', $A_p'/\Delta x$, which is estimated at about 0.7×10^3 cm g^{-1} muscle. For this estimate, the reflection coefficient was taken to be close to 1. Recent studies (Wolf and Watson, 1989) estimate the reflection coefficient for glucose in muscle capillaries to be 0.5, and this would then give a value of $A_p'/\Delta x$ of about 0.3×10^3 cm g^{-1} muscle. Further uncertainties concern the question of whether the pore is a simple channel or whether its permeability properties are determined by a fibre matrix (Adamson and Michel, 1993).

In the present study, we have obtained measurements of the capillary surface area, S(c), of the length of endothelial junctions, L(enj), and of the flux rate of glucose from the plasma, $\dot{M}_{CHO}$(iv), at different exercise intensities (Weber *et al.* 1996*b*). These data are obtained for total musculature in dogs and goats. Without any assumptions, we can calculate from these data the flux densities across the capillary endothelial surface, $\dot{M}_{CHO}(iv)/S(c)$, and across the unit length of endothelial junctions, $\dot{M}_{CHO}(iv)/L(enj)$.

The net flux rate across the endothelium is:

$$\dot{M}_{CHO}(iv) = p \times S(c) \times \overline{\Delta M}_{gluc}, \quad (1)$$

where the permeability coefficient $p=D(A_p'/\Delta x)/S(c)$, D is the diffusion coefficient, $\overline{\Delta M}_{gluc}$ is the molar transendothelial glucose concentration difference needed to drive the flux rate and S(c) is the capillary surface area. Taking values for $A_p'/\Delta x$ and D from the literature (Pappenheimer, 1953; Crone and

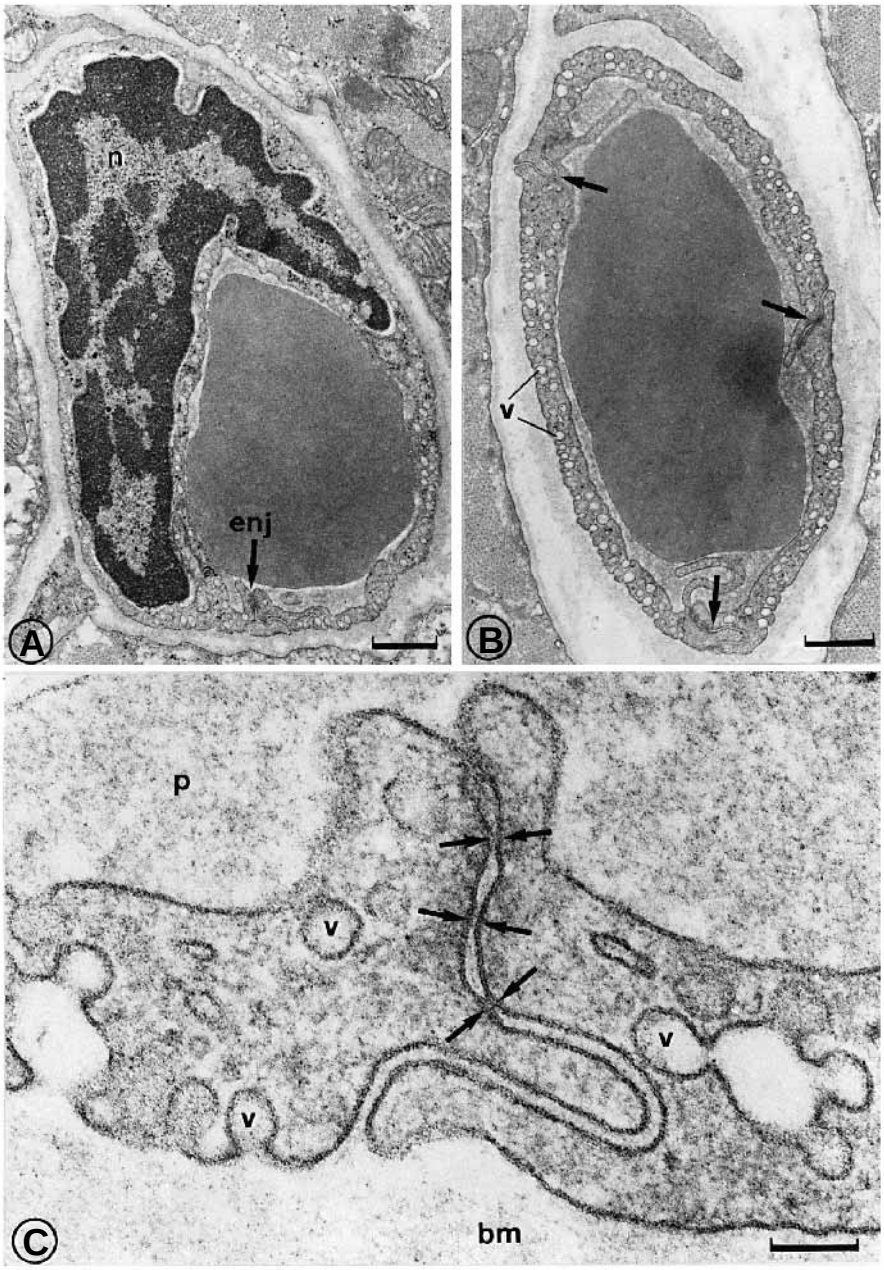

Fig. 3. Electron micrographs of capillaries in dog triceps muscle. (A) Capillary cross section with endothelial cell nucleus (n) and one endothelial junction (enj). (B) Cross section showing three endothelial junctions (arrows). (C) An endothelial junction, shown at higher magnification, extends as a tortuous slit connecting plasma (p) to the basement membrane (bm). Note that the plasma membranes are closely apposed in three points (arrows) corresponding to the ridges seen on freeze–fracture preparations. Plasmalemmal vesicles or caveolae (v) are shown in B and C. Scale bars, A, B, 0.5 μm; C, 0.1 μm.

Levitt, 1984; Curry, 1984), we can estimate $\overline{\Delta M}_{gluc}$. The uncertainties about the model conditions of the capillary pore system preclude, at present, further model calculations on the basis of our morphometric data.

How fatty acids are transferred from the plasma to the interstitial space is open to debate. Fatty acids are lipid-soluble and can therefore traverse the endothelial plasma membranes. In contrast, fatty acids are solubilized in plasma and interstitial fluid by binding to albumin so that an alternative pathway would be the passage of albumin-bound fatty acids through the small or large pore systems at the endothelial junctions.

The sarcolemma as an exchange barrier

Glucose transfer across the sarcolemma is driven by concentration differences, but it is facilitated by glucose transporters, GLUT-1 and GLUT-4, the latter being regulated in response to exercise and insulin (see Mueckler, 1994, for a review). The morphometric parameter for glucose uptake into the muscle cell is the sarcolemmal surface area, S(sl); the (variable) density of GLUT transporters will modify the glucose diffusion coefficient across the sarcolemma. The occurrence of fatty acid transporters in the sarcolemma is still controversial; fatty acids may well diffuse freely across the lipid layer of cell membranes (Glatz *et al.* 1988; Stremmel *et al.* 1992).

In order to assess the role of the sarcolemma in substrate transfer, we will calculate flux densities per unit surface area of the sarcolemma. We predict that the glucose flux density $\dot{M}_{CHO}$(iv)/S(sl) is proportional to the density of GLUT transporters in the sarcolemma and to the glucose

concentration difference between the interstitial space and the myocytoplasm, which are not known precisely. The corresponding flux density for fatty acids $\dot{M}_{FFA}(iv)/S(sl)$ is proportional to the specific conductance of the membrane for fatty acids and to the concentration difference for fatty acids between the interstitium and the myocytoplasm, which will depend on the binding coefficients of the fatty-acid-binding proteins.

Materials and methods

Animals and physiological studies

The study was carried out on tissue samples of three adult female pygmy goats (*Capra hircus*, M_b 21.0±0.3 kg) and three adult female Labrador dogs (M_b 23.7±1.0 kg; means ± S.E.M.). These animals were the subjects of the physiological studies discussed in the previous papers (Roberts *et al.* 1996; Weber *et al.* 1996*a*,*b*). The surgical procedures, training schedule, measurement of $\dot{M}_{O_2max}$ and exercise protocols of the animals are described in detail in Roberts *et al.* (1996).

In short, the animals were trained to run on an inclined treadmill (goats 18 %, dogs 29 % inclination) at different speeds until their $\dot{M}_{O_2}$ was reproducible at each speed. After determination of their individual maximal molar oxygen consumption rate ($\dot{M}_{O_2max}$), the animals were trained for three different exercise protocols, namely 2 h at 40 % $\dot{M}_{O_2max}$, 1 h at 60 % $\dot{M}_{O_2max}$ and 16 min at 85 % $\dot{M}_{O_2max}$. At each exercise intensity, glucose and fat oxidation rates were determined by means of indirect calorimetry (Roberts *et al.* 1996), and the supply of glucose and fatty acids from vascular and intracellular sources was estimated by means of infusions of [^{3}H]glucose and of [^{14}C]palmitate, respectively (Weber *et al.* 1996*a*,*b*).

Within approximately 24 h of the last physiological experiment, the animals were killed with an overdose of pentobarbital. Tissue samples were taken as described below and processed for morphometric studies. Additional samples were frozen for biochemical and histochemical studies. Whole-body muscle mass was determined by carefully dissecting and weighing the entire skeletal musculature of half the carcass of each animal. Head muscles were excluded since they do not serve locomotion.

Muscle tissue sampling, fixation and processing

The morphometric techniques have two refinements over our previous studies (Conley *et al.* 1987): (1) the muscle samples were obtained using a whole-body random sampling procedure (Hoppeler *et al.* 1984); and (2) the tissue blocks were sectioned using an isotropic uniform random (IUR) method so as to obtain unbiased estimates of all membrane areas despite different degrees of preferred orientation.

For each animal, 15 whole-body random samples of the skeletal musculature were collected within 90 min *post mortem* following a stratified volume-weighted sampling strategy described previously (Hoppeler *et al.* 1984). Thin longitudinal muscle strips were fixed in a 6.25 % solution of glutaraldehyde in 0.1 mol l^{-1} sodium cacodylate buffer (adjusted to 430 mosmol l^{-1} with NaCl); the total osmolarity of the fixative was 1150 mosmol l^{-1}, pH 7.4. The samples were subsequently cut into smaller blocks and stored in the same solution at 5 °C for about 1 month before further processing. After rinsing overnight in 0.1 mol l^{-1} sodium cacodylate buffer, the blocks were postfixed for 2 h in a 1 % solution of osmium tetroxide and block-contrasted with 0.5 % uranyl acetate. After dehydration with ethanol, they were embedded in Epon in moulds with a hemispherical bottom to ensure random orientation of the tissue blocks. The resulting Epon sticks were re-embedded in flat moulds from which blocks were cut out at directions determined by a system of random numbers representing spatial probabilities, thus providing IUR sections.

Morphometric analysis

The parameters of importance for this study are: the volume of capillaries, V(c), and of plasma, V(p), the surface area of capillary endothelium, S(c), and of sarcolemma, S(sl), the size of muscle fibres estimated from their mean cross-sectional fibre area, $\bar{a}$(f), and the volume of mitochondria in muscle fibres, V(mt), related to the muscle fibre volume, V(f), as reference parameter. The measurements were obtained using stereological methods which had to account for two major problems: (1) mitochondria, capillaries and muscle fibres differ in size and spacing, and (2) fibres and capillaries show different degrees of anisotropic orientation in space. These problems were overcome (1) by using a cascade sampling strategy with different magnifications (Weibel, 1979; Cruz-Orive and Weibel, 1981, 1990), and (2) by obtaining isotropic uniform random (IUR) sections of the muscle samples (e.g. Fig. 2), which permitted the unbiased estimation of all morphometric parameters using standard stereological methods (Weibel, 1979, 1980).

For the stereological analysis, IUR sections from each sample were subsampled in a Philips 300 transmission electron microscope at three different magnification levels, using sections of different thickness for reasons of image contrast and resolution. Micrographs were recorded on 35 mm film and examined in a projection device yielding a 10× secondary magnification; for calibration purposes, a carbon grating replica was recorded on each film.

Level I: low magnification

From each of the 15 random samples, IUR sections of about 100 nm thickness were picked up on 100 mesh copper grids covered with a carbon-coated Parlodion film and contrasted with lead citrate and uranyl acetate. From one section of each sample, 10 micrographs were taken at a final magnification of about 2800× on the morphometric screen. On these micrographs, we counted capillary and muscle fibre profiles according to the forbidden line rule (Gundersen, 1978); the mean number of muscle fibres counted per section was 28 for dogs and 36 for goats, and the mean number of capillaries per section was 76 for dogs and 56 for goats, with large variations depending on the orientation of the sectioning plane. By point

counting, we estimated the fraction of the section covered by muscle fibres as a reference parameter. This allowed the estimation of capillary length to fibre length ratio, J_J(c,f), capillary length density, J_V(c,f), and mean fibre cross-sectional area, $\bar{a}$(f), according to the following formulae:

$$J_J(\mathrm{c,f}) = Q(\mathrm{c})/Q(\mathrm{f}), \quad (2)$$

$$J_V(\mathrm{c,f}) = 2Q(\mathrm{c})/[P(\mathrm{f}) \times k_2 \times d^2], \quad (3)$$

$$\bar{a}(\mathrm{f}) = [P(\mathrm{f}) \times k_2 \times d^2]/[2Q(\mathrm{f})], \quad (4)$$

where Q is the number of transections of capillaries (c) or fibres (f), P(f) is the number of test points falling on muscle fibres, and k and d are the characteristics of the test system defining test line length in relation to test point number (Weibel, 1979).

We also attempted to determine, at this magnification, the volume density of interstitium, V_V(int,f), but this is not a reliable measurement because the interstitial spaces are distorted by tissue preparation and fixation.

Level II: intermediate magnification

From the same blocks, ultrathin sections of 50–70 nm thickness were cut, picked up on 200 mesh copper grids and again contrasted with lead citrate and uranyl acetate. On one of these sections per sample, 10 micrographs were taken at a final magnification of about 13 000× on the morphometric screen. At this magnification, the capillary and sarcolemmal surface area per fibre volume, S_V(c,f) and S_V(sl,f), were estimated by means of point and intersection counting. For calculation of the area of the small pore system of the capillary wall, we estimated the length of the endothelial cell junctions L(enj) by counting the number of junction profiles per capillary profile, Q(enj)/Q(c). The length of the endothelial junctions was then derived by multiplying this ratio by the estimated capillary length density:

$$L_V(\mathrm{enj,f}) = [Q(\mathrm{enj})/Q(\mathrm{c})] \times J_V(\mathrm{c,f}). \quad (5)$$

Owing to blood loss and shrinkage, the degree of capillary filling was artificially diminished in this immersion-fixed material, so that the volume density of capillaries V_V(c,f) could not be determined reliably by morphometry. Assuming that parameters such as capillary length density and surface area of capillary basement membrane are little influenced by these changes and regarding capillaries as cylindrical structures of diameter d(c), we calculated V_V(c,f) from the capillary surface area per fibre volume S_V(c,f) and the capillary length density J_V(c,f). We first estimated the mean radius r(c) of a cylindrical capillary whose circumference b is determined by dividing the surface area of the basement membrane by capillary length:

$$S_V(\mathrm{c,f})/J_V(\mathrm{c,f}) = b = 2\pi r(\mathrm{c}). \quad (6)$$

With this, we calculated the volume density of expanded cylindrical capillaries as:

$$V_V(\mathrm{c,f}) = \pi[r(\mathrm{c})]^2 \times J_V(\mathrm{c,f}). \quad (7)$$

Since there was no statistically significant difference between dogs and goats in the estimation of the mean capillary diameter $2r$(c) (see Table 1), we used the overall mean value for $2\bar{r}$(c) of 4.82 μm for both species.

Level III: high magnification

From each of these ultrathin sections, another set of 10 micrographs was taken at a final magnification of about 25 000× on the morphometric screen for the determination of mitochondrial volume density, V_V(mt,f).

Calculations and statistical analyses

The stereological calculations resulted in ratio estimates obtained by means of a program called STEPone (Humbert *et al.* 1990) with the 15 random samples for each animal taken together as one entity. From these ratio estimates, muscle-mass-specific values were calculated, assuming the muscle volume to be equal to the fibre volume and with a muscle density of 1.06 g ml^{-1} (Hoppeler *et al.* 1987).

To compare the two species groups, the mean of the three individual values per group and the standard error of the mean were calculated for each parameter. The Mann–Whitney U-test, a nonparametric test for group comparison, was used for statistical analyses, and a P value of 0.05 was interpreted as a statistically significant difference.

Results

Muscle mass and main physiological results

This study differs from previous ones with respect to the frame of reference for morphometric and physiological data: all data are expressed relative to muscle mass rather than to body mass. Although the animals of the two species groups were quite closely matched with respect to their body mass M_b, their whole-body skeletal muscle masses M_m differed more (Table 1). The relative muscle mass per M_b was 37 % in the dogs and only 26 % in the goats, giving a dog:goat ratio of 1.42. Since it is the skeletal muscles that are the main consumers of both oxygen and substrates during exercise, we decided to relate all our structural data to total muscle mass rather than to body mass. Likewise, we expressed all functional data with respect to muscle mass (Tables 1, 3). Thus, $\dot{V}_{O_2max}/M_b$ of 2.43 ml kg^{-1} s^{-1} in the dog and 1.10 ml kg^{-1} s^{-1} in the goat converted into $\dot{V}_{O_2max}/M_m$ of 6.67 ml kg^{-1} s^{-1} and 4.3 ml kg^{-1} s^{-1}, respectively, so that the dog:goat ratio was reduced from 2.21 to 1.55 (Table 1).

Morphometric results

As shown in Table 1, the mean muscle fibre cross-sectional area $\bar{a}$(f) was significantly larger in dogs than in goats (1549 and 1232 μm^2, respectively), whereas the sarcolemmal surface area per fibre volume S_V(sl,f) did not differ between the two species.

The capillary length density J_V(c,f) was 1.39 times larger in the dogs than in the goats, and the relative capillary surface area S_V(c,f) showed a dog:goat ratio of 1.54 (Table 1). The

Table 1. *Basic physiological and morphometric data of the animals used in this study*

Parameters	Dogs	Goats	Dog:goat ratio	Significance
M_b (kg)	23.7±1.0	21.0±0.3	1.13	*
M_m/M_b (%)	36.5±0.9	25.7±1.2	1.42	*
$\dot{V}_{O_2max}/M_b$ (ml O_2 kg^{-1} s^{-1})	2.43±0.047	1.10±0.045	2.21	*
$\dot{V}_{O_2max}/M_m$ (ml O_2 kg^{-1} s^{-1})	6.67±0.167	4.30±0.231	1.55	*
$\bar{a}$(f) (μm^2)	1549±94	1232±76	1.26	*
S_V(sl,f) (cm^2 cm^{-3})	1158±63	1257±65	0.92	NS
J_J(c,f) (cm cm^{-1})	2.72±0.21	1.54±0.13	1.76	*
J_V(c,f) (m cm^{-3} = mm^{-2})	1750±59	1255±84	1.39	*
S_V(c,f) (cm^2 cm^{-3})	279±33	181±13	1.54	*
2r(c) (μm)	5.04±0.43	4.60±0.09	1.10	NS
V_V(c,f) (%)	3.19±0.11	2.29±0.15	1.39	*
Q(enj)/Q(c)	1.71±0.03	1.76±0.04	0.97	NS
L_S(enj,c) (cm cm^{-2})	1096±85	1221±27	0.90	NS
V_V(mt,f) (%)	8.64±0.20	4.12±0.40	2.10	*
S_V(om,f) (cm^2 cm^{-3})	8819±400	5157±440	1.71	*

$\dot{V}_{O_2max}$ is the value calculated for the animals included in the morphometric study.

Capillary volume density per fibre V_V(c,f) and capillary diameter 2r(c) are calculated from capillary surface and length densities according to equations 6 and 7.

M_b and M_m are body and muscle mass, respectively.

J_V, S_V and V_V are length, surface area and volume densities of components (capillary, c; sarcolemma, sl; mitochondria, mt; outer mitochondrial membrane, om) per muscle fibre volume (f).

Q(enj)/Q(c) is number of endothelial junctions per capillary profile; L_S(enj,c) is length of endothelial junctions per endothelial surface area. $\bar{a}$(f) is mean cross-sectional muscle fibre area; J_J(c,f) is capillary per fibre length ratio.

Values are means ± S.E.M. (N=3).

Asterisks indicate a significant difference between the two species (P<0.05); NS, not significant.

capillary volume density V_V(c,f) calculated according to equation 7, and hence V(c)/M_m, showed a dog:goat ratio of 1.39 (Tables 1, 2).

Erythrocyte, V(ec), and plasma, V(p), volumes per muscle mass were calculated from the capillary volume and the venous haematocrit (45.0 % for the dogs and 25.3 % for the goats) (Table 2). The erythrocyte volume is related to oxygen transport and the plasma volume to substrate transport. The muscle-mass-specific erythrocyte volume V(ec)/M_m was significantly higher in dogs than in goats by a factor of 2.50, whereas no difference was found with respect to the plasma volume (ratio 1.02).

The mean number of endothelial junction profiles per capillary profile, Q(enj)/Q(c), was similar for dogs and goats, namely 1.71 and 1.76 (Table 1); the length of endothelial junctions per unit muscle mass, L(enj)/M_m, calculated from this parameter and the capillary length density, J_V(c,f), was 2829 and 2081 km kg^{-1} in dogs and goats respectively (Table 2).

In Table 2, the most relevant morphometric parameters are expressed per unit muscle mass. Note that the volume of plasma and the surface area of sarcolemma do not differ between dogs and goats, whereas the capillary erythrocyte volume and the capillary surface area as well as the length of the endothelial junction lines are larger in the aerobic species (dog).

Discussion

Microvasculature and the supply of O_2 and substrates

Maximal O_2 consumption was 2.2 times greater in the dogs than in the goats of this study when related to total body mass (Table 1; Roberts *et al.* 1996). Some of this difference was due to the greater relative muscle mass in the dog, but when maximal O_2 consumption is related to muscle mass it is still 1.55 times greater in the dog (Table 1). This significant difference correlates with a proportionally larger mitochondrial volume, as discussed in detail in Vock *et al.* (1996). All the O_2 required by the cell must be supplied from the capillaries, specifically from the erythrocytes, and so it is noteworthy that the volume of capillary erythrocytes per unit muscle mass was larger in the dogs than in the goats.

The focus of this series of studies was on the supply of substrates, carbohydrate and fatty acids, to the muscle cells and their mitochondria. In the physiological studies (Weber *et al.* 1996*a*,*b*), we reported that the total consumption rate of both carbohydrate and fatty acids at their respective maximal fluxes

Table 2. *Capillary and muscle fibre parameters per unit muscle mass calculated from data in Table 1*

Parameters	Dogs	Goats	Dog:goat ratio	Significance
$V(c)/M_m$ (ml kg^{-1})	30.1±1.02	21.6±1.44	1.39	*
Hct ven. (%)	45.0±2.4	25.3±1.8	1.78	*
$V(ec)/M_m$ (ml kg^{-1})	13.58±1.19	5.43±0.24	2.50	*
$S(ec)/M_m$ (m^2 kg^{-1})	17.93±1.57	9.34±0.41	1.92	*
$V(p)/M_m$ (ml kg^{-1})	16.5±0.29	16.2±1.39	1.02	NS
$S(c)/M_m$ (m^2 kg^{-1})	26.3±3.15	17.1±1.18	1.54	*
$S(sl)/M_m$ (m^2 kg^{-1})	109.2±6.0	118.6±6.2	0.92	NS
$S(om)/M_m$ (m^2 kg^{-1})	831.9±37.8	486.5±41.6	1.71	*
$V(mt)/M_m$ (ml kg^{-1})	81.5±1.9	38.9±3.8	2.10	*
$V(c)/V(mt)$ (ml ml^{-1})	0.369±0.005	0.560±0.026	0.66	*
$V(ec)/V(mt)$ (ml ml^{-1})	0.166±0.011	0.143±0.017	1.16	NS
$J(c)/M_m$ (km kg^{-1})	1651±56	1184±79	1.39	*
$L(enj)/M_m$ (km kg^{-1})	2829±101	2081±98	1.36	*

Red cell volume, $V(ec)$, is calculated from capillary volume, $V(c)$, venous haematocrit, Hct ven., and the surface of the red cells, $S(ec)$, by multiplying red cell volume by the surface area/volume ratio of 1.32 m^2 m^{-3} for dogs and 1.72 m^2 m^{-3} for goats.

$V(p)$ is plasma volume and $V(mt)$ is mitochondrial volume; $S(c)$, $S(sl)$ and $S(om)$ are the surface areas of capillaries, sarcolemma and outer mitochondrial membranes, respectively; $J(c)$ and $L(enj)$ are the lengths of capillaries and of endothelial cell junctions, respectively; M_m, muscle mass.

Values are means ± S.E.M., N=3.

Asterisks indicate a significant difference between the two species (P<0.05); NS, not significant.

was proportional to maximal O_2 consumption. In contrast, the flux rates from capillaries were similar in dogs and goats, despite a greater overall rate of substrate consumption in the dog. The difference was compensated for by drawing more on the intracellular substrate stores, a point that will be discussed in greater detail by Vock *et al.* (1996). This suggests that the supply of substrate from the circulation is limited and the question arises of whether this limitation is due to the structural properties of the pathway from capillaries to the mitochondria in muscle cells.

Methodological problems and solutions

The first methodological problem was to find a suitable frame of reference for comparing a large set of structural and functional measurements obtained from two different species. It is customary in comparative physiology to normalize functional data to body mass. The same is done for morphometric data, but these are often collected on specific muscles only and extrapolated to the whole organism. This did not seem suitable for the present study. Since, during heavy exercise, over 90 % of fuel oxidation occurs in skeletal muscle, it appeared preferable to compare both functional and morphometric data with total muscle mass as frame of reference (Hoppeler *et al.* 1984). We estimated the total mass of locomotor muscles by dissection of all our animals and found that relative muscle mass, M_m/M_b, was 1.42 times greater in the dog, which compares well with a previous estimate (Hoppeler *et al.* 1987). Choosing this more appropriate frame of reference provided the opportunity to determine differences between the dog and goat that were not due simply to differences in total active muscle mass.

Having selected the entire locomotor musculature as the frame of reference, we used a sampling strategy to provide us with a volume-weighted stratified random sample of whole-body muscle. Since the physiological data relate to whole-body musculature, the functional and morphometric studies operate in an identical frame of reference.

Isotropic uniform random (IUR) sectioning of the samples is a further innovation. Because muscle cells and capillaries are anisotropic structures, the estimation of capillary and sarcolemmal surface areas, $S(c)$ and $S(sl)$, and of capillary length density, $J_V(c,f)$, by stereological methods depends upon the direction of the sectioning plane. In previous studies, we have used a model-based approach to estimate capillary length density from traditional capillary counts on transverse sections, assuming capillary geometry to be described by a Dimroth–Watson orientation distribution (Mathieu *et al.* 1983; Cruz-Orive *et al.* 1985). This technique cannot be used for estimating the surface area of sarcolemma. We therefore chose to sample the tissue using IUR sections (Weibel, 1980; Cruz-Orive and Weibel, 1990; Nyengaard and Gundersen, 1992). This approach provides unbiased estimates of all parameters of interest, such as capillary length density, $J_V(c,f)$, capillary surface area per fibre volume, $S_V(c,f)$, and the surface area per fibre volume of the sarcolemmal membranes, $S_V(sl,f)$, at the expense of sampling efficiency.

We could also estimate a new parameter from these sections: the length of the intercellular junction lines in capillary endothelium (Fig. 3), an important variable because it estimates the extent of the small pore system for solute flux between the plasma and the interstitial space, which appears to be located along the intercellular junctions.

Another problem was that the volume density of interstitium and of capillaries measured on these preparations could not be regarded as a reliable estimate. Immersion fixation leads to an artificial and erratic enlargement of the space between the muscle fibres (Hoppeler *et al.* 1987), while large connective tissue compartments may escape sampling. Hence, we decided *a priori* to disregard the contribution of interstitium and to regard the whole muscle mass as fibre mass. The appearance of capillaries in these preparations was likewise marred by artefacts, mostly because blood was lost as a consequence of the fixation procedure. Thus, capillary volume V(c) and morphometric capillary haematocrit could not be reliably estimated. By assuming that capillary length density and the surface area of the capillary basement membrane are not much affected by these artefacts, we could calculate the capillary volume density V_V(c,f) from the capillary length density and the capillary surface density as indicated in equations 6 and 7. The capillary diameter estimated by this procedure did not differ significantly between dogs and goats and averaged 4.82 μm. This value, determined on IUR sections, is close to the values found by Conley *et al.* (1987) using transverse sections under the same model assumptions.

Morphometric results

Capillary length density J_V(c,f) as well as capillary surface area per fibre volume S_V(c,f) were significantly larger for the dogs than for the goats [dog:goat ratios were 1.39 for J_V(c,f) and 1.54 for S_V(c,f)]. In consequence, we found the muscle-mass-specific capillary volume V(c)/M_m to be 1.39 times greater in the dogs than in the goats, which is in reasonable agreement with previous data (Conley *et al.* 1987).

The dog:goat ratio of capillary length to fibre length, J_J(c,f), was 1.76. Although this parameter is related to the capillary-to-fibre number ratio, N_N(c,f), customarily estimated, it cannot be readily compared with previous data. This is because N_N(c,f) is commonly estimated on transverse rather than IUR sections so that differences in the tortuosity of the capillaries are masked. The observed ratio means that there are more capillaries around one fibre in the dogs than in the goats, and that the greater blood supply in the dogs is not achieved by larger capillaries but by more or longer capillaries. This is again in good agreement with the results of Conley *et al.* (1987).

In our previous studies on adaptive variation, we compared dogs with goats, and horses or ponies with cattle, and arrived at the conclusion that the capillary erythrocyte volume was proportional to $\dot{M}_{O_2max}$ (Weibel *et al.* 1991, 1992). The dog:goat ratio for V(ec) to $\dot{M}_{O_2max}$ observed here does not support this conclusion. A closer look at the previous data set reported by Conley *et al.* (1987) reveals that a similar relationship to that described here for erythrocyte volume to $\dot{M}_{O_2max}$ was observed in the dog:goat comparison, whereas the match was good for ponies and calves, as it was for horses and steers (Weibel *et al.* 1991). The discrepancy may be related to the much smaller red cell size of the goats compared with that of the other five species, which have similar red cell sizes. This may warrant further investigation.

The surface areas of the consecutive barriers between the circulation and the mitochondria are of importance for oxygen and substrate delivery. As shown in Table 2, capillary, sarcolemmal and outer mitochondrial membrane surface areas (in $m^2\,kg^{-1}$ muscle mass) behave differently: the capillary and outer mitochondrial membrane surface areas S(c)/M_m and S(om)/M_m are significantly larger in dogs than in goats with similar ratios (1.54 and 1.71, respectively), whereas the sarcolemmal surface area S(sl)/M_m is the same in dogs and goats. The smallest interface is the capillary surface, the next larger one is the sarcolemmal surface, and the largest one is the last one in the transport pathway, the mitochondrial surface, so that the ratio S(c):S(sl):S(om), all expressed per M_m, is 0.24:1:8 in the dogs and 0.14:1:4 in the goats (Table 2).

Relationships between functional and structural data

We now relate the substrate flux data, reported in the companion studies (Weber *et al.* 1996*a*,*b*) and summarized in Table 3, to the structural properties of the supply pathway from capillary to muscle mitochondria. We do this by calculating flux densities, i.e. by dividing substrate flux rates by surface areas. This approach makes the assumption that all structural capacity is available at all work intensities. While this may not be the true situation, it is likely that the relative proportion of capillaries perfused at any work intensity is similar in dogs and goats. The most relevant finding of our functional studies is that only a small fraction of the amount of substrates oxidized is derived directly from intravascular substrate pools. At 40 % $\dot{M}_{O_2max}$, where total fatty acid oxidation rate is highest, the capillary plasma supplies 23 % and 27 % of the fatty acid oxidized in dogs and goats, respectively (Table 3; Weber *et al.* 1996*a*). The remainder is drawn from intracellular substrate stores. In the dog, fatty acid flux from vascular sources remains the same as exercise intensity increases, but it rises in the goat to reach the same value as in the dogs at 60 % $\dot{M}_{O_2max}$ (Table 3; Weber *et al.* 1996*a*). This represents the highest flux rate from vascular sources, and we shall therefore compare flux data from the capillary at this exercise intensity (Figs 4, 5). Although carbohydrate oxidation increases steadily with exercise intensity, the amount of carbohydrate drawn from plasma remains nearly constant, contributing 13 % of the total glucose oxidized at 85 % $\dot{M}_{O_2max}$ in the dog and 23 % in the goat (Table 3; Weber *et al.* 1996*b*). Fig. 4 compares the relevant morphometric parameters supporting O_2 and substrate supply from the capillaries to the mitochondria. The higher $\dot{M}_{O_2max}$/M_m in the dog is associated with a greater erythrocyte and mitochondrial volume. Supporting the functional observation that substrate supply from the blood is the same in dog and goat, we find that plasma volume and sarcolemmal surface area are also the same in the two species. The capillary surface area, however, is larger in the dog.

How are these findings related to the flux rates observed for the substrates? The critical limiting steps in the transfer of substrates from the plasma to the mitochondria in muscle cells occur at the barriers established by the endothelium and sarcolemma. In Table 3, we have calculated flux densities for

Table 3. *Molar oxidation rates and flux densities for the animals used in this study*

Parameters	Dogs	Goats	Dog:goat ratio
Oxygen at $\dot{M}_{O_2max}$			
$\dot{M}_{O_2}/M_m$ (μmol min^{-1} kg^{-1})	17 937	11 778	1.52
$\dot{M}_{O_2}/S$(c) (μmol min^{-1} m^{-2})	682	689	0.99
$\dot{M}_{O_2}/S$(sl) (μmol min^{-1} m^{-2})	164.3	99.3	1.65
Glucose at 85 % $\dot{M}_{O_2max}$			
$\dot{M}_{O_2}^{CHO}$(mt)/M_m (μmol min^{-1} kg^{-1})	12 236	8 603	1.42
$\dot{M}_{O_2}^{CHO}$(iv)/M_m (μmol min^{-1} kg^{-1})	1 619	1 981	0.82
$\dot{M}_{CHO}$(iv)/M_m (μmol min^{-1} kg^{-1})	270	330	0.82
$\dot{M}_{CHO}$(iv)/S(c) (μmol min^{-1} m^{-2})	10.3	19.3	0.53
$\dot{M}_{CHO}$(iv)/L(enj) (pmol min^{-1} m^{-1})	95.4	158.6	0.60
$\dot{M}_{CHO}$(iv)/S(sl) (μmol min^{-1} m^{-2})	2.45	2.78	0.88
Fatty acids at 40 % $\dot{M}_{O_2max}$			
$\dot{M}_{O_2}^{FFA}$(mt)/M_m (μmol min^{-1} kg^{-1})	4 575	3 233	1.42
$\dot{M}_{O_2}^{FFA}$(iv)/M_m (μmol min^{-1} kg^{-1})	1 074	879	1.22
$\dot{M}_{FFA}$(iv)/M_m (μmol min^{-1} kg^{-1})	46.7	38.2	1.22
$\dot{M}_{FFA}$(iv)/S(c) (μmol min^{-1} m^{-2})	1.77	2.24	0.79
$\dot{M}_{FFA}$(iv)/S(sl) (μmol min^{-1} m^{-2})	0.428	0.322	1.33
Fatty acids at 60 % $\dot{M}_{O_2max}$			
$\dot{M}_{FFA}$(iv)/M_m (μmol min^{-1} kg^{-1})	48.3	52.3	0.92
$\dot{M}_{FFA}$(iv)/S(c) (μmol min^{-1} m^{-2})	1.84	3.06	0.60
$\dot{M}_{FFA}$(iv)/S(sl) (μmol min^{-1} m^{-2})	0.442	0.441	1.00

Molar oxidation rates per unit muscle mass and flux densities for O_2, glucose (CHO) and free fatty acids (FFA) from intravascular sources (iv) across the capillary, S(c), and sarcolemmal surface, S(sl), as well as through the length of intercellular junctions, L(enj), calculated for the exercise intensities at which maximal rates were measured.

Functional data are calculated from mean values given in Weber *et al.* (1996*a*,*b*), expressed per kilogram muscle mass.

Abbreviations are defined in the text.

O_2 and the two substrates across these barrier surfaces. These values are also shown in Fig. 5 as a function of exercise intensity.

Glucose transfer through endothelium and sarcolemma

Endothelium

The flux density for glucose across the unit capillary surface, $\dot{M}_{CHO}$(iv)/S(c), is larger in the goat than in the dog. Both species draw about the same amount of glucose from the blood, but the goat's smaller capillary surface area causes the flux density through the endothelium to be nearly twice as high in the goat as in the dog (Table 3). This estimate, however, is not very relevant because it is well established that hydrophilic solutes such as glucose leave the capillary through pores in the endothelial cell junctions (Pappenheimer, 1953; Crone and Levitt, 1984). It is therefore more relevant to express glucose flux rate per unit length of intercellular junction. We found that the length of junction lines per unit surface area of capillary endothelium, L_S(enj,c), was not different between dogs and goats (Table 1). Accordingly, the flux density across the junctions is also some 60 % larger in the goats than in the dogs. Qualitative examination of intercellular junctions showed no difference between dog and goat; for lack of further information, we therefore assume that they do not differ in terms of their permeability characteristics.

The flux density across the junction lines is obtained without any assumptions about the pore system that mediates the transfer, but it is clearly not a satisfactory parameter because its physiological meaning is difficult to interpret. Little is known that can be applied to the present situation, although the structure of endothelial junctions has been extensively studied (Perry, 1980; Frøkjaer-Jensen, 1985; Adamson and Michel, 1993).

In order to overcome such difficulties, Pappenheimer (1953) introduced a compound parameter that assesses the permeability of the intercellular restricted pore system without the need to know the actual pore dimensions: the restricted pore area per unit path length, $A_p'/\Delta x$, discussed above in relation to equation 1. The value of $A_p'/\Delta x$ for glucose was estimated from physiological data obtained in the dog to be 0.7×10^3 cm g^{-1} muscle (Pappenheimer, 1953) or, if expressed per unit area of capillary surface area, 2.7 cm^{-1}. Multiplying this parameter by the diffusion coefficient for glucose, $D=0.91\times10^{-5}$ cm^2 s^{-1}, yields the permeability coefficient p of the capillary wall, which is thus estimated at 2.45×10^{-5} cm s^{-1}. These estimates were obtained on the perfused hindleg muscles of the dog, and we assume that they are applicable to the entire musculature in both the dog and the goat since there are no apparent differences in the fine structure of the capillary wall.

In equation 1, the flux rate for glucose from the capillary is expressed as the product of the permeability coefficient, the capillary surface area and the mean concentration gradient across the capillary wall. Since we have measured flux rate and capillary surface area in dogs and goats, we can now make use of this estimate of permeability to calculate the concentration gradient, which we find to be 0.7 mmol l^{-1} in the dog and 1.3 mmol l^{-1} in the goat. If we use newer estimates of the reflection coefficient based on a permeability model allowing for water-exclusive pores (Wolf and Watson, 1989), we calculate the mean concentration differences to be 1.5 and 2.7 mmol l^{-1} in dog and goat, respectively, which would mean that the concentration difference across the capillary wall is about one-quarter of the plasma concentration in both species. On the basis of a different approach and data set, Crone and Levitt (1984) estimated this concentration difference to be approximately 3 mmol l^{-1} during exercise, which is in reasonable agreement with our values.

Interstitium

From these data, we can attempt to estimate the interstitial glucose concentration, which is the driving force for glucose transfer across the sarcolemma. In the pericapillary region, the glucose concentration is the difference between the plasma concentration and the transendothelial glucose concentration gradient. At 85 % $\dot{M}_{O_2max}$, the plasma concentrations were

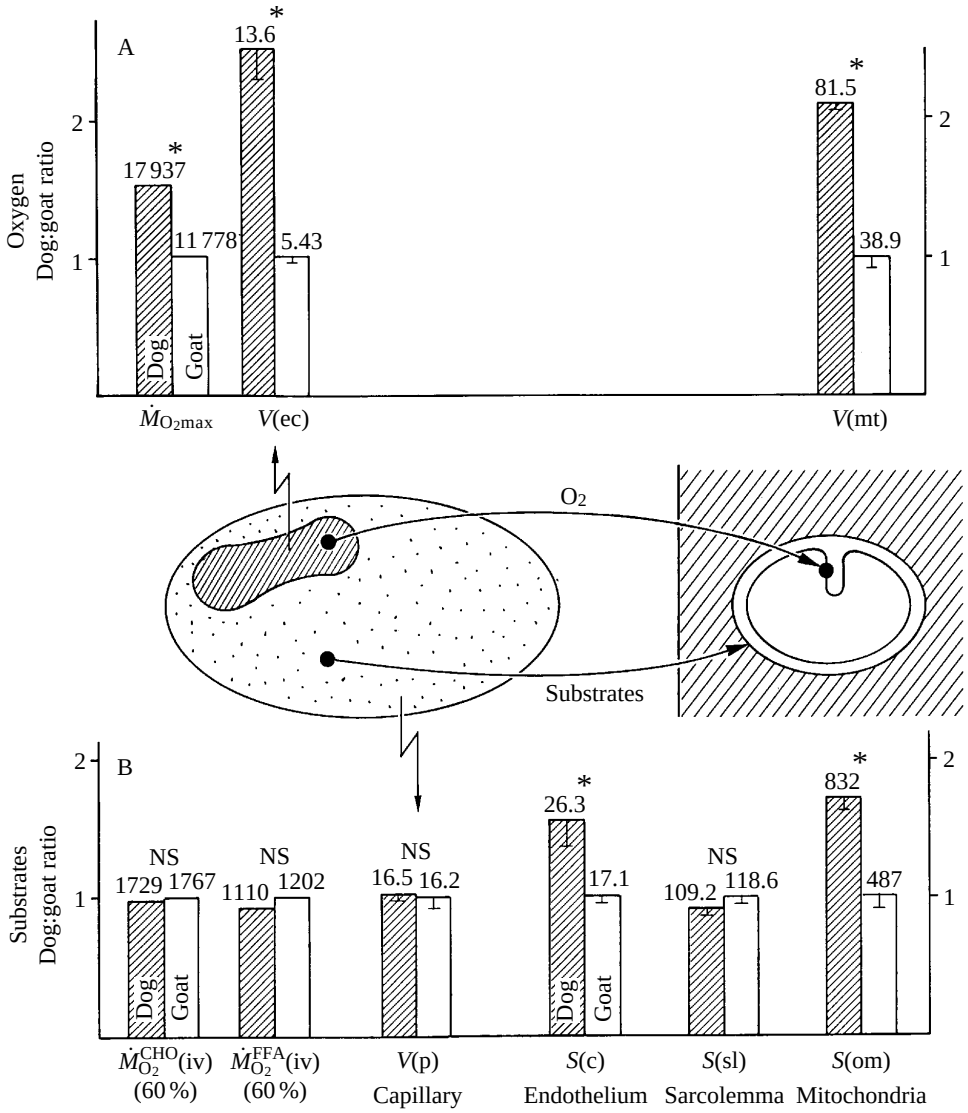

Fig. 4. Comparison of microvascular transport of oxygen (A) and substrates (B) to muscle cells with relevant morphometric parameters for capillaries and mitochondria. All data are expressed per kilogram muscle mass and plotted as dog:goat ratios; absolute values are given above each column. N=3 animals per group. Asterisks mark significant differences between groups (P<0.05). Physiological data are calculated from Weber *et al.* (1996*a*,*b*) for an exercise intensity of 60 % $\dot{M}_{O_2max}$, morphometric data are from Table 2, where symbols and units are given. $\dot{M}_{O_2}^{CHO}$(iv) and $\dot{M}_{O_2}^{FFA}$(iv) are molar oxidation rates of vascular glucose and fatty acids in oxygen equivalents per kilogram muscle mass ($\mu mol\,O_2\,kg^{-1}\,min^{-1}$).

found to be 5.4 and 11.5 mmol l^{-1} in the dog and goat, respectively (Weber *et al.* 1996*b*). From the above calculations, we estimate the transendothelial gradient to 1–2 mmol l^{-1} in the dog and 1.5–3 mmol l^{-1} in the goat. As a result, we estimate the pericapillary glucose concentration to be about 4 and 9 mmol l^{-1} in dog and goat, respectively. The driving force for delivering glucose from the capillary to the sarcolemma thus appears to be twice as large in the goat as in the dog.

As shown in Fig. 2A, capillaries are in contact with muscle fibres over a limited part of the sarcolemma so that most of the sarcolemmal surface is in contact with a narrow interstitial space 1–2 μm wide, which forms the pathway for distributing glucose derived from the capillary to the sarcolemmal membrane. The average distance between capillaries can be derived from the ratio of sarcolemmal surface area to the length of the capillaries, as reported in Table 2; it is 66 μm in the dog and 100 μm in the goat. As glucose diffuses from the capillary into this space, it passes along two sarcolemmal membranes which extract glucose at approximately the same rate of about 4×10^{-14} μmol $\mu m^{-2}\,s^{-1}$ in both species. This must be compared with a concentration gradient of the order of 4×10^{-12} μmol μm^{-3}, so that each μm^2 of sarcolemmal surface extracts about 1 % of the glucose contained in the interstitial space every second. As glucose spreads from the capillary, the concentration will therefore fall exponentially (cable function) as a function of the difference between diffusion along the surface and extraction. Since the half-distance between capillaries is 50 % longer in the goat than in the dog, this will result in a similar mean concentration of glucose at the sarcolemmal surface, lending further support to our calculations.

Sarcolemma

The transfer of glucose across the sarcolemma is driven by concentration differences across the membrane, but it is facilitated by the presence of carriers in the membrane. This is well documented for glucose transporters of the GLUT family (for a review, see Mueckler, 1994). Our physiological studies of glucose uptake refer to high activity levels at steady state

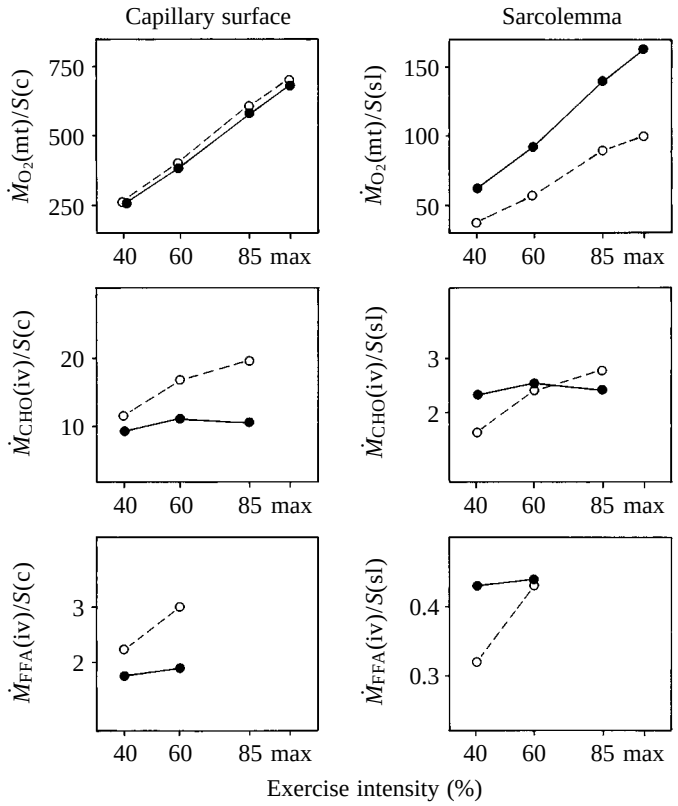

Fig. 5. Flux densities for oxygen, glucose and fatty acids at the capillary endothelial and sarcolemmal diffusion barriers in relation to exercise intensity. Physiological data are from Weber *et al.* (1996*a*,*b*) and morphometric data are from Table 2. Filled circles and solid lines indicate values for dogs (N=3); open circles and broken lines indicate values for goats (N=3). $\dot{M}_{O_2}$(mt) is molar oxygen consumption at a given exercise intensity (all O_2 coming from capillaries and serving oxidation in mitochondria). $\dot{M}_{CHO}$(iv) and $\dot{M}_{FFA}$(iv) are molar oxidation rates of vascular carbohydrates and fatty acids at given exercise intensities; max, maximum exercise intensity. S(c) and S(sl) are capillary and sarcolemmal surface areas, respectively.

(85 % $\dot{M}_{O_2max}$), so that we assume that all the available GLUT-4 transporters have been recruited (Goodyear *et al.* 1991). We have estimated the flux density of glucose per unit surface area of sarcolemmal membrane, $\dot{M}_{CHO}$(iv)/S(sl), and found it to be approximately 2.5 μmol m^{-2} min^{-1} in both goats and dogs (Table 3) and at all exercise intensities, with the exception of the lowest exercise level in the goat (Fig. 5). This suggests that the transfer capacity of the sarcolemmal membrane, or rather of its transporters, is saturated at all exercise intensities, certainly above 60 % $\dot{M}_{O_2max}$; since there is no evidence for large differences in the transmembrane glucose gradient between the two species, we may further conclude that the conductance of the sarcolemma for glucose, determined largely by the density and activity of GLUT transporters, is similar in both species. The observation that the flux density reaches an upper limit at rather low exercise intensities suggests that glucose uptake by the muscle cells is limited by the sarcolemma and that transfer properties are the same in dogs and goats. Accordingly, all the additional glucose required for running at higher speeds must be drawn from intracellular stores (Weber *et al.* 1996*b*); this will be discussed further in the next paper of this series (Vock *et al.* 1996).

Fatty acid transfer through endothelium and sarcolemma

Information on fatty acid transport from the blood to the muscle cells is scantier than that for glucose transport. The discussion of structure–function relationships in this limb of the substrate pathway is therefore of necessity incomplete.

Endothelium

Dogs and goats draw a similar amount of fatty acids from the plasma pool; as a consequence of the greater capillary surface area in dogs, the flux density of free fatty acids at the capillary surface, $\dot{M}_{FFA}$(iv)/S(c), is smaller than in the goats (Table 3). Little is known about the transfer of fatty acids across the endothelial barrier. As lipophilic substances, they can cross the lipoproteinaceous cell membranes and thus use the entire capillary surface for their transfer from plasma to interstitial fluid provided that there is an adequate transport mechanism within the cytoplasm of the endothelial cell. In contrast, fatty acids are bound to albumin in the plasma and also in the interstitial fluid. The estimated molecular diameter of albumin is about 4 nm and hence of the same order as the estimated pore size at the intercellular junctions. The reflection coefficient of albumin is 0.9 (Crone and Levitt, 1984) so that a small fraction can traverse the pore system. The fatty acid flux density at the capillary wall is about one-sixth of that of glucose (Table 3); considering that one albumin molecule binds about six fatty acid molecules, the flux of albumin across the pores would only have to be about one-fortieth of that of glucose to account for the transendothelial fatty acid transfer rate.

Sarcolemma

Fatty acid transport across the sarcolemmal membrane is not as well understood as that for glucose. Fatty acid transporter proteins have been postulated to occur in the sarcolemma, but this is still debated (Higgins, 1994). Fatty acids could well cross the lipophilic membranes without transporters. In this regard, it is of interest that the flux densities across the sarcolemma are similar for goats and dogs at the two exercise intensities where they were measured (Table 3), so that we conclude that the sarcolemma has the same conductivity for fatty acids in dogs and goats and that, if there are any fatty acid transporters in these membranes, their density and activity would be about the same in both species.

Fluid spaces

The pathway for fatty acid supply from the capillaries to the mitochondria also involves transport steps across aqueous spaces, the interstitial space and the sarcoplasm, where fatty acids are transferred bound either to albumin in the interstitial space or to fatty-acid-binding proteins in the sarcoplasm (Glatz *et al.* 1988). There are no data on these fatty-acid-binding proteins in dogs and goats which would allow us to discuss

possible differences in these transfer steps, except for the observation that albumin has a 1.5-fold higher fatty acid binding capacity in the dogs than in the goats (McClelland *et al.* 1994). However, the distances for fatty acid transfer between the endothelium and the sarcolemma and between the sarcolemma and the mitochondria are shorter in dogs than in goats because dogs have a higher surface density of both capillaries and mitochondria (Table 2).

Conclusions

This morphometric study was undertaken to test whether the structures constituting the pathway of vascular substrate supply limit substrate flux from capillaries to muscle mitochondria. Our findings suggest the following.

(1) The higher maximal oxygen uptake of the dogs' muscle cells is matched by a greater structural capacity: a higher capillary erythrocyte volume and surface area, and a higher mitochondrial content of the muscle cells.

(2) Substrate extraction is the same in dogs and goats when related to plasma volume because the larger haematocrit in dog blood results in a reduced plasma content (giving similar plasma volumes in the two species). This is related to the finding that the substrate flux rates from the intravascular pools are similar in dogs and goats.

(3) The transfer of glucose from the plasma to the interstitial fluid is assumed to occur through the small pore system at the endothelial intercellular junctions. We estimate that this pore system does not introduce a major resistance to glucose flux and that the concentration difference across these channels is about one-quarter of the plasma concentration in both species. Similar conclusions are drawn with respect to fatty acid transfer.

(4) The surface area of sarcolemma is invariant between dogs and goats, and the flux densities for glucose and fatty acids across the sarcolemma reach the same level in both species and do not increase with increasing exercise intensity. This suggests (a) that the density and/or activity of glucose (and putative fatty acid) transporters are similar in dogs and in goats, and (b) that these transport systems are already saturated at the lower exercise intensities.

(5) The sarcolemma constitutes the major resistance in the pathway for substrates and is thus the chief limiting design factor for substrate supply from vascular sources to muscle cells.

(6) The structure of the supply pathway from the blood to the mitochondria in muscle cells is designed primarily for steady O_2 supply up to maximal rates of oxidation. This pathway is inadequate to supply glucose and fatty acids from vascular pools at the high rates required during strenuous exercise. Capillary substrate supply through this pathway occurs at low rates, predominantly during periods of rest or low activity, thus building intracellular stocks of carbohydrate and lipid that can be exploited during exercise.

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