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DESIGN OF THE OXYGEN AND SUBSTRATE PATHWAYS

IV. PARTITIONING ENERGY PROVISION FROM FATTY ACIDS

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Summary

This paper quantifies the fluxes of fatty acids through the pathways supplying muscle mitochondria with oxidative fuel in exercising dogs and goats. We used continuous infusions of 1-[^{14}C]palmitate and indirect calorimetry to measure fatty acid supply from two sources: the circulation and the triglyceride stores within the muscle cells. Our goal was to determine maximal flux through these two branches of the lipid pathway as key functional parameters for testing the principle of symmorphosis, i.e. that structural capacity is quantitatively matched to functional demand in the oxidative substrate pathways. It is under these rate-limiting conditions that we predict that all of the structural capacity will be used. Maximal rates of fatty acid oxidation were reached at low exercise intensities of 40 % $\dot{M}_{O_2max}$. Fatty acids from the circulation supplied only a small fraction (15–25 %) of the total fat oxidized under these conditions. Although dogs were able to oxidize circulatory fatty acids faster than goats, maximal rates were not in proportion to the 2.2-fold difference in aerobic capacity between the two species. Dogs compensated for their relatively lower use of circulatory fatty acids by oxidizing more triglycerides from lipid droplets in their muscle cells. We conclude that fatty acids from intramuscular triglyceride stores are a major source of fuel during maximal rates of lipid oxidation. Furthermore, it is this branch of the fatty acid pathway that is adapted to the large difference in aerobic capacity between dogs and goats.

Key words: metabolism, exercise, fat oxidation, dog, goat, symmorphosis.

Introduction

In mammals, aerobic exercise is fuelled mainly by carbohydrates and lipids (Weber, 1992). In the second paper of this series, we have shown that oxidation rates of both substrates are scaled with aerobic capacity: when running at the same percentage of $\dot{M}_{O_2max}$, highly aerobic dogs use 2.2 times more carbohydrates and lipids than sedentary goats (Roberts *et al.* 1996). Similarly, endurance-trained rats (Brooks and Donovan, 1983) and humans (Coggan *et al.* 1990) are able to oxidize lipids faster than untrained individuals. These metabolic fuels are supplied to locomotory muscle mitochondria from nearby intramuscular stores and from remote stores *via* the circulation. Even though maximal rates of lipolysis and mitochondrial lipid oxidation are both scaled with aerobic capacity (Weber *et al.* 1993; McClelland *et al.* 1994; Roberts *et al.* 1996), the relative roles of these two supply pathways may not be the same in highly aerobic and sedentary animals. For example, endurance training increases the relative contribution of intramuscular fatty acid stores (Hurley *et al.* 1986).

Here, we focus our attention on the pathways supplying fatty acids to muscle mitochondria and determine maximal rates of fatty acid oxidation from intramuscular and circulatory sources. Our model of the fatty acid pathway is presented in Fig. 1, where the steps relevant to our discussion have been numbered (see also Taylor *et al.* 1996). Step 1 indicates the rate of fatty acid release from adipose tissue triglyceride stores into the blood compartment. Flux at this step is the rate of appearance of fatty acids into the circulation or R_{aFFA} (the abbreviation commonly used in whole-organism metabolic studies). In the present paper, we use R_{aFFA} to be consistent with the normal terminology used in the field of metabolic biochemistry, but in subsequent papers of this series we use different abbreviations as we integrate functional and morphometric parameters for our test of symmorphosis. Step

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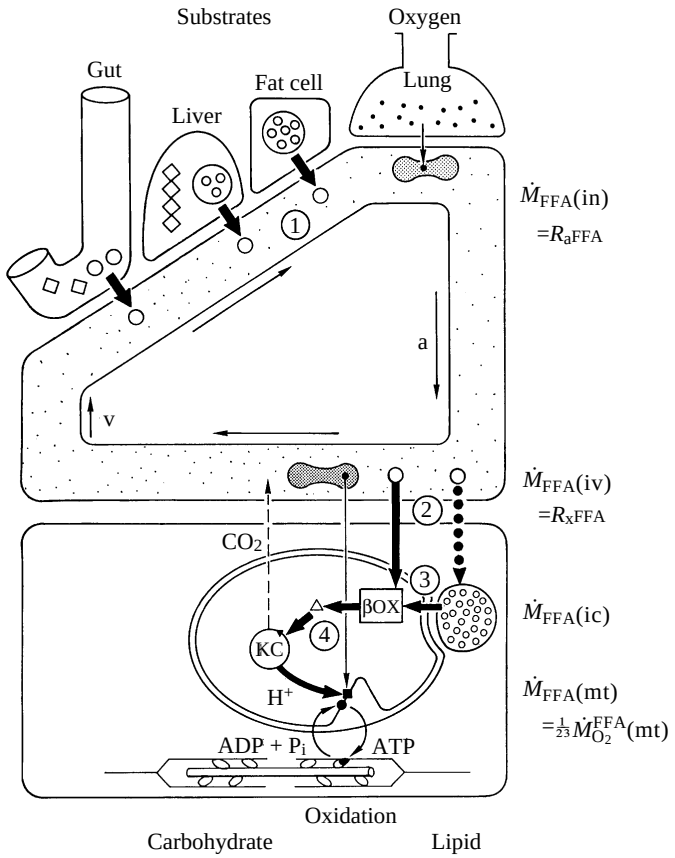

Fig. 1. Model of the lipid pathway indicating (heavy arrows) fatty acid supply (circles) to locomotory muscle mitochondria from the circulation and from intramuscular lipid droplets. Note that the supply of fatty acids to lipid droplets (dotted arrow) is temporally split from the oxidation of fatty acids. The O_2 pathway is marked with dots and thin arrows, CO_2 discharge is marked with broken arrows. The triangle marks acetyl-CoA. a, arterial; v, venous; in, 'inflow'; iv, intravascular; ic, intracellular (intramuscular); mt, mitochondrial; βOX, β-oxidation; KC, Krebs cycle; H^+, reducing equivalents; black square, terminal oxidase in the inner mitochondrial membrane. Step 1, rate of appearance of fatty acids in the circulation; $R_{aFFA}=\dot{M}_{FFA}$(in). Step 2, rate of removal of circulatory fatty acids; $R_{xFFA}=\dot{M}_{FFA}$(iv). Step 3, rate of oxidation of intramuscular fatty acid stores; $\dot{M}_{FFA}$(ic). Step 4, rate of total fatty acid oxidation in mitochondria; $\dot{M}_{FFA}(mt)=\dot{M}_{FFA}(iv)+\dot{M}_{FFA}(ic)$, which is equal to 1/23 of O_2 consumption induced by fatty acid oxidation in mitochondria $\dot{M}_{O_2}^{FFA}$(mt).

1 is R_{aFFA}, which we will call $\dot{M}_{FFA}$(in) in subsequent papers of this series. The rate of oxidation of fatty acids from the circulation, R_{xFFA}, is step 2 in the model and is called $\dot{M}_{FFA}$(iv). Step 3 is the rate of fatty acid oxidation from intramuscular triglyceride stores, $\dot{M}_{FFA}$(ic). Finally, step 4 is the rate of total mitochondrial fatty acid oxidation, $\dot{M}_{FFA}$(mt), which is equal to the sum of the oxidation rates of circulatory plus intramuscular fatty acids. Our model does not include volatile fatty acids for the goat, because they do not represent a significant source of energy in exercising ruminants; the most abundant volatile fatty acid is acetate (Bergman, 1990), which only provides less than 2 % of the total energy to the muscles of exercising sheep (Jarrett *et al.* 1976).

In this paper, our goal was to obtain data for testing the hypothesis of symmorphosis by measuring maximal rates of fatty acid oxidation from the circulation and from intramuscular triglyceride stores in exercising dogs and goats. These are the major functional parameters of the fatty acid pathway which we will correlate with corresponding structural components in subsequent papers in this series.

Materials and methods

Three female dogs (23.7±1.0 kg; mean ± S.E.M.) and three female goats (21.0±0.3 kg) were used for these experiments. They were the same individuals used in the previous papers with the exception of one goat (Roberts *et al.* 1996; Weber *et al.* 1996). These same animals were also used for all the morphometric measurements performed in subsequent papers of this series (Vock *et al.* 1996*a*,*b*). The treadmill, training conditions and surgical procedures to move the carotid arteries to a subcutaneous position have been described previously (Roberts *et al.* 1996). Two exercise protocols were selected for the present experiments: 1 h at 40 % $\dot{M}_{O_2max}$ and 1 h at 60 % $\dot{M}_{O_2max}$. These particular intensities were chosen because maximal lipid oxidation can be elicited at these work rates (Martin *et al.* 1993; McClelland *et al.* 1994; Weber *et al.* 1993).

Catheterization

One day before measuring fatty acid kinetics, a sterile PE-50 catheter was fed through the jugular vein into the pulmonary artery under local lidocaine anaesthesia for infusion of labelled palmitate. The exact location of the catheter was confirmed by connecting it to a pressure transducer and by monitoring pressure changes as it was advanced in the vessel. To sample blood, a second sterile PE-50 catheter was placed into the aorta *via* the exteriorized carotid artery. These infusion and sampling sites have been shown to provide the most accurate measurement of fatty acid fluxes (Jensen *et al.* 1988). Between experiments, catheters were kept patent by flushing with pure saline every second day and by keeping them filled with heparinized saline. Particular care was taken to avoid injecting any heparin for at least 24 h before an isotope infusion.

Labelled palmitate infusions

All experiments were carried out on an inclined indoor motorized treadmill at 18 % (goat) and 29 % (dog) incline at an ambient temperature of 6–14 °C. Food was withheld for 24 h before each infusion. To prepare the infusates, goat and dog albumin were purchased from Sigma (St Louis, MO, USA) and they were delipidated with isopropylether (Sham and Knowles, 1976) immediately before each infusion. A saline solution of delipidated albumin was mixed with 1-[^{14}C]palmitate (Amersham, Arlington Heights, IL, USA; specific activity 2.06 GBq mmol^{-1}) and sonicated. This particular fatty acid was

selected because it is the best representative of the total fatty acid pool in both species used in this study (McClelland *et al.* 1995). Furthermore, the use of palmitate labelled on carbon 1 to quantify fatty acid kinetics in humans and in animal models has been thoroughly validated (Miles *et al.* 1987; Spitzer, 1975; Vranic, 1975).

While the trained animal was at rest, a continuous infusion of labelled palmitate was started using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA). No priming dose was needed because the total fatty acid pool is small (Wolfe, 1984). However, the CO_2 pool was primed with 2×10^5 disints min^{-1} kg^{-1} of ^{14}C-labelled sodium bicarbonate to ensure that steady CO_2 specific activities were reached rapidly. Infusions were initiated 70 min before exercise to allow isotopic steady state for both CO_2 and palmitate to be reached and to measure resting kinetics. The animals were trained to stand quietly on the treadmill during the pre- and post-exercise resting periods. The exact infusion rate was determined separately for each experiment by counting a sample of the infusate. At rest and during recovery, mean infusion rates were 55 595±3802 disints min^{-1} kg^{-1} min^{-1} for goats (N=6) and 59 647±7692 disints min^{-1} kg^{-1} min^{-1} for dogs (N=6). During exercise at both intensities, infusion rate was doubled for both species to reduce fluctuations in specific activity (Levy *et al.* 1989; Molina *et al.* 1990; Weber *et al.* 1990, 1993). Successive measurements on the same animal were always separated by at least 10 days, and a blood sample was taken before each infusion to confirm that no residual activity from the previous experiment was present. Throughout the infusions, the animals wore a loose-fitting mask, allowing the continuous measurements of O_2 consumption and CO_2 production as described previously (Roberts *et al.* 1996).

Blood sampling and analyses

During the isotope infusions, a series of 3 ml blood samples was taken *via* the aortic catheter to measure CO_2 and palmitate specific activities. They were drawn 50, 60 and 70 min after the start of infusion at rest, and after 20, 40 and 60 min of exercise. The blood used for CO_2 analyses was collected anaerobically. $^{14}CO_2$ activity was determined immediately after sampling as follows: 1 ml of whole blood was rapidly placed in a tightly capped 25 ml Warburg flask containing 2 ml of water, 100 µl of sodium heparin and one drop of octanol. The CO_2 was released by injecting 1 ml of citric acid (0.3 mol l^{-1}) through the flask cap, and it was subsequently trapped in a well containing 200 µl of Solvable (New England Nuclear, Boston, MA, USA) on a paper filter. The flasks were gently shaken for 1 h at room temperature before counting the filters in Formula 989 scintillation fluid (New England Nuclear, Boston, MA, USA). 200 µl of whole blood was used to measure CO_2 content in duplicate using a modified Tucker method as described by Cameron (1971).

The remaining blood was centrifuged and used for the measurement of palmitate specific activity. 0.5 ml of plasma was mixed with the same volume of an EDTA/HCl solution (100 mmol l^{-1} EDTA, 1 mol l^{-1} HCl, 1:9 v/v). Margaric acid (17:0) was then added as an internal standard before extracting the fatty acids with a 2:1 mixture of isopropylether and isopropanol. Three-quarters of the organic extract was counted in ACS II scintillation fluid (Amersham) to determine palmitate activity. Preliminary experiments with [^{3}H]oleate showed that, under these conditions, 97–99 % of total plasma fatty acid activity was extracted. Also, preliminary infusions of U-[^{14}C]palmitate (Amersham) in resting and exercising animals showed that all the plasma fatty acid activity was present in palmitate, as determined by counting high-performance liquid chromatography (HPLC) fractions of the different fatty acids separately; no measurable ^{14}C was incorporated into acids other than palmitate. All counting was performed on a Beckman LS1801 scintillation counter with external quench correction.

Palmitate concentration was determined by HPLC on the rest of the organic extract according to the method of Miles *et al.* (1987). Briefly, after derivatization of the fatty acids to their phenacyl esters with bromoacetophenone (Wood and Lee, 1983), they were separated on a Spherisorb column (ODS, 5 m, 4.5 mm×250 mm, C18 reverse phase, Beckman) with acetonitrile:water:methanol (83:17:5) at a flow rate of 2 ml min^{-1}, using an ultraviolet detector at 242 nm. Plasma free fatty acid (FFA) concentration was calculated as the total surface area of all fatty acid peaks using the margaric acid peak (17:0) as a reference.

Terminology, calculations and statistics

The model presented in Fig. 1 shows the relevant functional steps of the fatty acid pathway. The rate of appearance of palmitate in the circulation, R_a palmitate, was calculated using the steady-state equation of Steele (1959), as previously validated for fatty acid kinetics (Miles *et al.* 1987). The rate of appearance of fatty acids was then determined by dividing R_a palmitate by the fractional contribution of palmitate to total FFA concentration. Circulatory palmitate oxidation (R_x palmitate) was calculated by dividing the rate of $^{14}CO_2$ production (=CO_2 specific activity × $\dot{M}_{CO_2}$) by palmitate specific activity (Wolfe, 1984). No correction was made for incomplete CO_2 excretion because oxidation rates were only calculated during exercise, when CO_2 retention becomes negligible (Wolfe *et al.* 1984). The rate of circulatory fatty acid oxidation (R_xFFA) was calculated by multiplying R_aFFA by the fraction of palmitate oxidized (=R_x palmitate/R_a palmitate). Rates of total lipid oxidation were calculated from $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ using the equations of Frayn (1983) as described previously (Roberts *et al.* 1996). The rate of fatty acid oxidation from triglyceride stores in the muscle cells was calculated by subtracting the rate of oxidation of circulating fatty acids from the rate of total lipid oxidation. Results were analysed using two- or three-way analyses of variance with replication (ANOVA) and Tukey's test after arcsine transformation when values were percentages. All values presented are means and standard errors.

Results

Plasma fatty acid concentration and specific activity

Plasma palmitate concentration (Fig. 2A) and total fatty acid concentration (Table 1) were higher in dogs than in goats ($P<0.01$), but did not change significantly between rest and exercise or throughout exercise ($P>0.05$). Overall, palmitate specific activity (Fig. 2B) was higher in goats than in dogs ($P<0.0001$). The twofold increase in infusion rate between rest and exercise maintained specific activity constant in dogs. In goats, however, a significant increase in specific activity was observed, and all the exercise values were higher than the rest values ($P<0.01$). For both species, palmitate specific activity remained in steady state throughout exercise (Fig. 2B, $P>0.05$).

Fatty acid kinetics

During exercise, steady-state conditions prevailed in both species because no changes in palmitate concentration, specific activity or flux were observed over time (Fig. 2A–C). During exercise, the rate of appearance of plasma palmitate (R_a palmitate) was much higher in dogs than in goats ($P<0.001$), but no difference between the species was found at rest (Fig. 2C). Exercise caused a significant increase of R_a palmitate in dogs ($P<0.05$), but not in goats. Table 1 shows the effect of exercise on the rate of appearance of fatty acids (R_aFFA). R_aFFA was much higher in dogs than in goats during exercise ($P<0.01$), but not at rest or at 20 min of 60 % $\dot{M}_{O_2max}$. In dogs, all R_aFFA exercise values were higher than rest values ($P<0.05$). No differences were found between the R_aFFA responses measured at 40 and at 60 % $\dot{M}_{O_2max}$ ($P>0.2$).

Fatty acid oxidation

Rates of circulatory palmitate (R_x palmitate) and total FFA oxidation (R_xFFA) during exercise are presented in Fig. 3 and Table 1, respectively. R_x palmitate and R_xFFA were always much higher in dogs than in goats ($P<0.02$). For both species, these oxidation rates were stable throughout exercise ($P>0.05$),

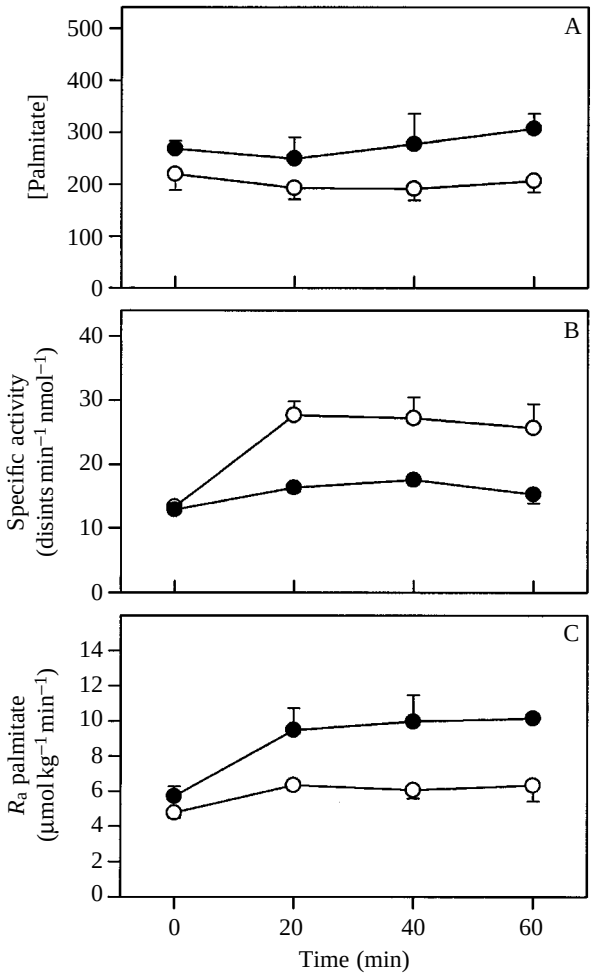

Fig. 2. Plasma concentration (A), specific activity (B) and rate of appearance (C) of palmitate (R_a palmitate) in trained goats (open circles) and trained dogs (filled circles) before (time 0) and during treadmill running at 40 % $\dot{M}_{O_2max}$. The rate of 1-[^{14}C]palmitate infusion was doubled during exercise. Values are means ± S.E.M. (N=3).

Table 1. *Mean values for plasma fatty acid concentration [FFA], rate of appearance of fatty acids [*$R_aFFA = \dot{M}_{FFA(in)}$*], rate of plasma fatty acid oxidation [*$R_xFFA = \dot{M}_{FFA(iv)}$*] and dog:goat ratio for* R_xFFA

			40 % $\dot{M}_{O_2max}$			60 % $\dot{M}_{O_2max}$		
		Rest	20 min	40 min	60 min	20 min	40 min	60 min
[FFA]	Dog	1004±33	911±143	1029±217	1189±116	806±166	1088±61	–
(nmol ml^{-1})	Goat	917±125	725±111	735±124	779±101	729±157	779±173	706±121
R_aFFA = $\dot{M}_{FFA}$(in)	Dog	21.4±2.0	34.8±4.8	37.0±5.4	39.4±1.3	26.9±1.6	36.0±6.2	–
(μmol kg^{-1} min^{-1})	Goat	20.1±1.2	23.7±1.0	23.0±2.4	23.8±3.8	28.1±6.0	24.6±4.2	25.2±3.0
R_xFFA = $\dot{M}_{FFA}$(iv)	Dog	–	15.6±2.6	17.4±5.6	18.1±5.6	17.3±4.2	17.9±4.1	–
(μmol kg^{-1} min^{-1})	Goat	–	8.8±2.6	9.9±1.6	10.8±2.4	13.9±1.4	14.4±1.5	15.0±1.0
R_xFFA	Dog:goat		1.77	1.75	1.68	1.24	1.24	–

Measurements were carried out on trained dogs (N=3) and trained goats (N=3) running on a treadmill at exercise intensities of 40 % and 60 % $\dot{M}_{O_2max}$.

Values are means ± S.E.M.

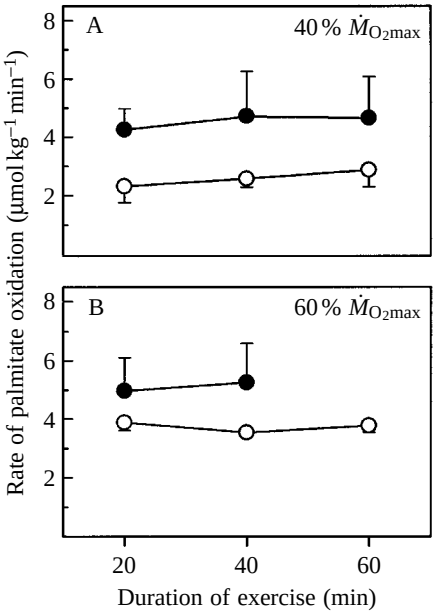

Fig. 3. Rate of palmitate oxidation in trained goats (open circles) and trained dogs (filled circles) during treadmill running at 40 % (A) and 60 % $\dot{M}_{O_2max}$ (B). Values are means ± S.E.M. (N=3).

and no significant difference was found between exercise at 40 and at 60 % $\dot{M}_{O_2max}$ (P>0.05). The dog:goat ratios for circulatory free fatty acid oxidation (R_{xFFA}) are also given in Table 1. Values ranged between 1.77 at 40 % $\dot{M}_{O_2max}$ and 1.24 at 60 % $\dot{M}_{O_2max}$. Table 2 shows mean molar rates of oxygen consumption per unit body mass for total metabolism, total lipid oxidation and circulating fatty acid oxidation in μmol O_2 kg^{-1} min^{-1}. These mean values are used in subsequent papers of this series dealing with the structural elements involved in fatty acid transport.

The contribution of circulating fatty acid oxidation to whole-animal oxidation $\dot{M}_{O_2}$ is shown in Fig. 4 for both exercise intensities. Circulating fatty acid oxidation accounted for a larger fraction of total oxidation in goats than in dogs (P<0.0001), and the relative importance of this oxidative substrate was slightly higher at an exercise intensity of 40 % than at 60 % $\dot{M}_{O_2max}$ (P<0.02).

Table 2. *Molar oxidation rates per unit body mass in dogs and goats running at two exercise intensities (40 and 60 % $\dot{M}_{O_2max}$)*

	Dogs		Goats	
	40 % $\dot{M}_{O_2max}$	60 % $\dot{M}_{O_2max}$	40 % $\dot{M}_{O_2max}$	60 % $\dot{M}_{O_2max}$
$\dot{M}_{O_2}/M_b$	2590	3420	987	1750
$\dot{M}_{O_2}^{FFA}$(mt)/M_b	1614	747	649	532
$\dot{M}_{O_2}^{FFA}$(iv)/M_b	392	405	226	309

Oxidation rates are in μmol O_2 kg^{-1} min^{-1} for total metabolic rate ($\dot{M}_{O_2}/M_b$), total lipid oxidation [$\dot{M}_{O_2}^{FFA}$(mt)/M_b] and circulating fatty acid oxidation [$\dot{M}_{O_2}^{FFA}$(iv)/M_b].

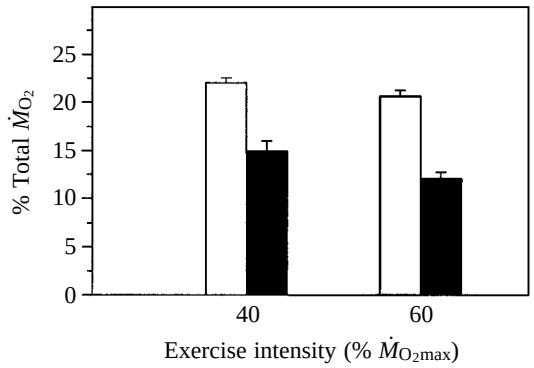

Fig. 4. Relative contribution of circulatory fatty acid oxidation to total $\dot{M}_{O_2}$ in goats (open bars) and dogs (filled bars) during treadmill running at 40 and 60 % $\dot{M}_{O_2max}$. Values are means ± S.E.M. (N=3).

Relative contribution of the different oxidative fuels

The percentage contributions of muscle triglycerides, circulating fatty acids and total carbohydrates to total metabolic rate at each exercise level are presented in Fig. 5. The relative importance of total lipid oxidation decreased as exercise intensity increased and the opposite was true for total carbohydrate oxidation (see Roberts *et al.* 1996). The contribution of circulating fatty acid oxidation to total oxidation was higher in goats than in dogs at both exercise intensities. Fig. 6 summarizes the relative contributions of circulatory and intramuscular substrate oxidation to total lipid and total carbohydrate oxidation under conditions of maximal lipid oxidation (60 % $\dot{M}_{O_2max}$; this study) and maximal carbohydrate utilization (85 % $\dot{M}_{O_2max}$; Weber *et al.* 1996). The percentage contributions of circulating fatty acids and circulating glucose were much higher in goats than in dogs (P<0.05). Conversely, the percentage contributions of muscle triglycerides and muscle glycogen were higher in dogs than in goats (P<0.05). Circulating fatty acids accounted for a larger

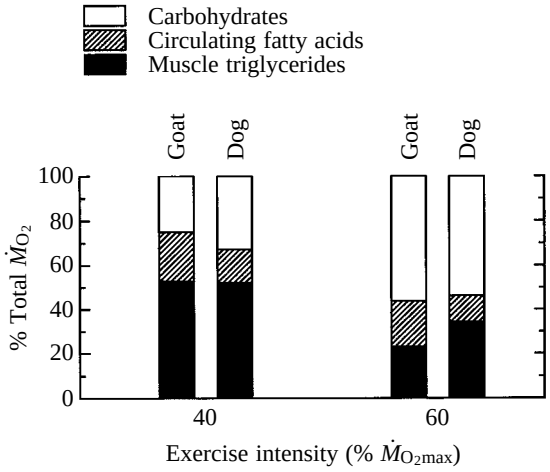

Fig. 5. Oxidative fuel utilization in goats and dogs exercising at 40 and 60 % $\dot{M}_{O_2max}$. Cumulative percentage contributions of muscle triglyceride stores, circulating fatty acids and total carbohydrate oxidation to total oxygen consumption (100 % $\dot{M}_{O_2}$) are indicated.

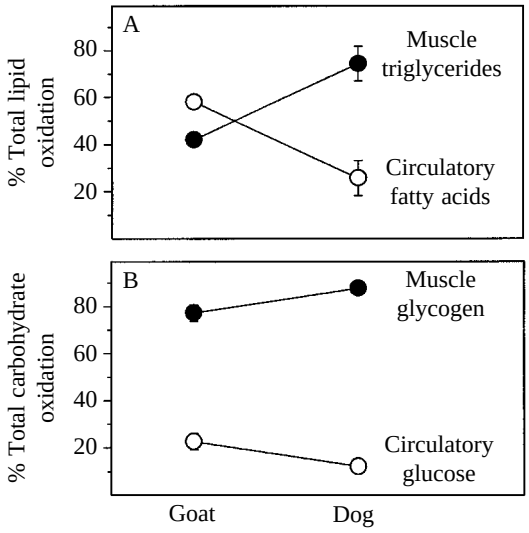

Fig. 6. Changes in the relative contributions of circulatory and intramuscular substrate utilization as a function of aerobic capacity [$\dot{M}_{O_2max}$(dog)/$\dot{M}_{O_2max}$(goat)=2.2] under conditions of maximal rates of fuel oxidation (85 % $\dot{M}_{O_2max}$ for carbohydrates and 60 % $\dot{M}_{O_2max}$ for fats). Increased aerobic capacity causes a decrease in the relative use of circulating substrates and a corresponding increase in the use of intramuscular energy stores for both lipids (A) and carbohydrates (B). Standard errors of the means are only indicated when larger than the symbols. Data for B are taken from Weber *et al.* (1996).

fraction of total lipid oxidation than muscle triglycerides in goats (P<0.0001), but the opposite was true for dogs (P<0.05). Muscle glycogen accounted for a much larger fraction of total carbohydrate oxidation than did circulating glucose in both species (P<0.001).

Discussion

The importance of intracellular stores

The direct pathway from lipid droplets in muscle cells to mitochondria (Fig. 1) is by far the most important source of fatty acids contributing to maximal rates of lipid oxidation during exercise. The indirect pathway (from adipose tissue, liver and gut into the circulation; transfer in the blood to the muscle capillaries; transfer across the endothelial cells, interstitial space and sarcolemma into the muscle cells; and then through the cytosol) to mitochondria (as presented in Fig. 1) supplies only about a quarter of the maximal rate of fatty acid oxidation in the mitochondria of dogs and about half in goats. The goat value is consistent with the results of Martin *et al.* (1993) for whole-body measurements on humans, but lower than values reported by Turcotte *et al.* (1992) for the leg during a one-legged dynamic knee extension exercise (75 % of fatty acid flux oxidized). Finally, the maximal rates of circulatory fatty acid oxidation are only slightly different between species (Table 2), despite the 2.2-fold difference in total fat oxidation and total metabolic rate. Again, as we saw for carbohydrates in the previous paper (Weber *et al.* 1996), the difference is made up for by the higher rates of oxidation of intramuscular stores in the dog.

Circulating fatty acids as oxidative fuel

Maximal rates of oxidation of fatty acids from both circulatory and intramuscular origin are reached at a low exercise intensity (Fig. 4; Roberts *et al.* 1996). In the mammals studied to date, submaximal work is known to cause an increase in fatty acid release from adipose tissue to the circulation, together with an increase in uptake by locomotory muscles (Ahlborg *et al.* 1974; Paul and Issekutz, 1967; Romijn *et al.* 1993; Shaw *et al.* 1975; Wolfe *et al.* 1990). These changes are responsible for augmenting rates of circulatory fatty acid oxidation by muscle mitochondria (Martin *et al.* 1993; Turcotte *et al.* 1992).

In both dogs and goats, circulatory fatty acid oxidation is not increased beyond the maximal level achieved at 40 % $\dot{M}_{O_2max}$. The endurance-adapted dogs reach a significantly higher maximal rate of circulatory fatty acid oxidation (Table 2), but the relative contribution of circulatory fatty acids to total metabolism (Fig. 4) and to total lipid oxidation (Fig. 6) is much lower than in goats.

This pattern is similar to the changes in fat oxidation rates that occur during endurance training, where reliance on muscle triglycerides also increases (Holloszy *et al.* 1986; Hurley *et al.* 1986; Jansson and Kaijser, 1987; Martin *et al.* 1993). These data support the hypothesis that the oxidation of circulatory fatty acids is limited by functional or structural barriers, and it has been suggested that maximal rates of lipid transport in aqueous plasma (McClelland *et al.* 1994; Weber, 1992) and lipid translocation across cell membranes (Weber, 1988) could play an important role in setting the upper limit for rates of oxidation of circulating free fatty acids. Producing ATP through the oxidation of muscle triglyceride stores instead of circulatory fatty acids makes sense because it circumvents these constraints.

Fatty acid transport through the circulation and cytosol is mediated by plasma albumin (Andersson, 1979) and intracellular fatty-acid-binding proteins (FABPs) (Clarke and Armstrong, 1989) in the same way that oxygen transport depends on the presence of haemoglobin and myoglobin. Therefore, albumin and FABP availability, together with their capacity to bind fatty acids, affect the maximal rates of circulatory fatty acid oxidation. Observations that maximal fatty acid binding capacity is higher for dog than for goat albumin (McClelland *et al.* 1994) and that FABP concentration is increased in chronically stimulated rat muscle (Kaufmann *et al.* 1989) strongly support this hypothesis.

In concluding this discussion of fatty acid flux through the various branches of the fatty acid pathway (Fig. 1), it is important to emphasize that steady-state conditions were established for all the parameters of lipid metabolism measured in this study. Palmitate concentration, palmitate specific activity, R_a palmitate (Fig. 2), CO_2 specific activity and palmitate oxidation rate (R_x palmitate) (Fig. 3) did not change significantly throughout exercise. Derived parameters for total

fatty acids (concentration, R_{aFFA} and R_{xFFA}) were consequently also in steady state (Tables 1, 2). Isotopic steady states for CO_2 and palmitate were also observed in other studies where equivalent protocols for priming the bicarbonate pool and for labelled palmitate infusion were used (Martin *et al.* 1993; Turcotte *et al.* 1992). The fact that exercise steady states were reached in this study, as well as in previous experiments on humans, indicates that the intra- and extracellular pools of palmitate and CO_2 were in dynamic equilibrium during the palmitate infusions.

Conclusions

The following observations have been made.

(1) Energy supply rates from circulating fatty acids and from intramuscular triglyceride stores do not increase as exercise intensity is increased above 40 % $\dot{M}_{O_2max}$.

(2) About half of all fatty acids oxidized during maximal rates of lipid oxidation are provided from the circulation in goats, but only one-quarter in dogs.

(3) Dogs can release fatty acids from adipose tissue faster (higher maximal R_{aFFA}), and use circulatory fatty acids faster (higher R_{xFFA}) than sedentary goats, but not in proportion to the 2.2-fold difference in aerobic capacity; thus, their relative use of circulatory fatty acids is lower.

(4) Dogs compensate for the shortfall in fatty acid supply from the circulation by using relatively more triglycerides from their muscle lipid droplets than do goats.

This pattern of higher reliance on intramuscular fuel reserves, together with a lower relative utilization of circulatory fuels, is also found for carbohydrates (Weber *et al.* 1996). Furthermore, in the sixth paper of this series (Vock *et al.* 1996*a*), we show that muscle triglyceride stores are 2.3 times larger in dogs than in goats. We conclude that the structural design of the direct fatty acid pathway from intramuscular lipid stores to mitochondria must provide 2–3 times the transport capacity in dogs compared with goats and that the design of the more convoluted pathway involving the circulation is not adjusted to differences in aerobic capacity.

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