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 $M_O^\text{max}$, 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 $R_{\text{aFFA}}$ (the abbreviation commonly used in whole-organism metabolic studies). In the present paper, we use $R_{\text{aFFA}}$ 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

**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-[14C]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 $% M_O^\text{max}$. 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.
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. 1996a,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 % \( M_{O2,max} \) and 1 h at 60 % \( M_{O2,max} \). 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 isopropyl ether (Sham and Knowles, 1976) immediately before each infusion. A saline solution of delipidated albumin was mixed with 1-[14C]palmitate (Amersham, Arlington Heights, IL, USA; specific activity 2.06 GBq mmol\(^{-1}\)) and sonicated. This particular fatty acid was 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.

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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 CO2 pool was primed with 2×10^5 disintegrations min^-1 kg^-1 of 14C-labelled sodium bicarbonate to ensure that steady CO2-specific activities were reached rapidly. Infusions were initiated 70 min before exercise to allow isotopic steady state for both CO2 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 infusedate. At rest and during recovery, mean infusion rates were 55.59±3802 disintegrations min^-1 kg^-1 min^-1 for goats (N=6) and 59.64±7692 disintegrations 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 O2 consumption and CO2 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 CO2 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 CO2 analyses was collected anaerobically. 14CO2 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 CO2 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 CO2 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 isopropanol and isopropanol. Three-quarters of the organic extract was counted in ACS II scintillation fluid (Amersham) to determine palmitate activity. Preliminary experiments with [3H]oleate showed that, under these conditions, 97–99 % of total plasma fatty acid activity was extracted. Also, preliminary infusions of U-[14C]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 14C 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 bromoacetylaphenone (Wood and Lee, 1983), they were separated on a Spherisorb column (ODS, 5 μm, 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, $R_p$, 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 $R_p$, palmitate by the fractional contribution of palmitate to total FFA concentration. Circulatory palmitate oxidation ($R_o$, palmitate) was calculated by dividing the rate of 14CO2 production (=$CO_2$ specific activity × $M_{CO_2}$) by palmitate specific activity (Wolfe, 1984). No correction was made for incomplete CO2 excretion because oxidation rates were only calculated during exercise, when CO2 retention becomes negligible (Wolfe et al. 1984). The rate of circulatory fatty acid oxidation ($R_{FFA}$ FFA) was calculated by multiplying $R_o$, FFA by the fraction of palmitate oxidized (= $R_p$, palmitate/$R_o$, palmitate). Rates of total lipid oxidation were calculated from $M_O$, and $M_{CO_2}$ 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 ($R_a$ FFA). $R_a$ FFA was much higher in dogs than in goats during exercise ($P<0.01$), but not at rest or at 20 min of 60% $M_O^{\text{max}}$. In dogs, all $R_a$ FFA exercise values were higher than rest values ($P<0.05$). No differences were found between the $R_a$ FFA responses measured at 40 and at 60% $M_O^{\text{max}}$ ($P>0.2$).

Fatty acid oxidation

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

Table 1. Mean values for plasma fatty acid concentration [FFA], rate of appearance of fatty acids [$R_{a\text{FFA}} = M_{\text{FFA(in)}}$], rate of plasma fatty acid oxidation [$R_{x\text{FFA}} = M_{\text{FFA(iv)}}$] and dog:goat ratio for $R_{x\text{FFA}}$

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

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% $M_O^{\text{max}}$. Values are means ± S.E.M. (N=3).
and no significant difference was found between exercise at 40 and at 60 % \( M\dot{O}_2 \) \(_{\max} \) \((P>0.05)\). The dog:goat ratios for circulatory free fatty acid oxidation \((R_{\text{FFA}})\) are also given in Table 1. Values ranged between 1.77 at 40 % \( M\dot{O}_2 \) \(_{\max} \) and 1.24 at 60 % \( M\dot{O}_2 \) \(_{\max} \). 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 \( \mu \text{mol} \text{O}_2 \text{kg}^{-1} \text{min}^{-1} \). 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 \( M\dot{O}_2 \) 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 % \( M\dot{O}_2 \) \(_{\max} \) \((P<0.02)\).

**Table 2. Molar oxidation rates per unit body mass in dogs and goats running at two exercise intensities (40 and 60 % \( M\dot{O}_2 \) \(_{\max} \)).**

<table>
<thead>
<tr>
<th></th>
<th>Dogs (40% M\dot{O}<em>2 ) (</em>{\max}), 60% ( M\dot{O}<em>2 ) (</em>{\max})</th>
<th>Goats (40% M\dot{O}<em>2 ) (</em>{\max}), 60% ( M\dot{O}<em>2 ) (</em>{\max})</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M\dot{O}_2 /M_b )</td>
<td>2590, 3420</td>
<td>987, 1750</td>
</tr>
<tr>
<td>( M\dot{O}<em>2 ) (</em>{\text{FFA}}) (_{\text{(mt)}}/M_b )</td>
<td>1614, 747</td>
<td>649, 532</td>
</tr>
<tr>
<td>( M\dot{O}<em>2 ) (</em>{\text{FFA}}) (_{\text{(iv)}}/M_b )</td>
<td>392, 405</td>
<td>226, 309</td>
</tr>
</tbody>
</table>

Oxidation rates are in \( \mu \text{mol} \text{O}_2 \text{kg}^{-1} \text{min}^{-1} \) for total metabolic rate \((M\dot{O}_2/M_b)\), total lipid oxidation \([M\dot{O}_2 \) \(_{\text{FFA}}\) \(_{\text{(mt)}}/M_b]\) and circulating fatty acid oxidation \([M\dot{O}_2 \) \(_{\text{FFA}}\) \(_{\text{(iv)}}/M_b]\).

**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 % \( M\dot{O}_2 \) \(_{\max} \); this study) and maximal carbohydrate utilization (85 % \( M\dot{O}_2 \) \(_{\max} \); 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
for carbohydrates in the previous paper (Weber et al. 1993). The goat value is consistent with the results of Martin et al. (1993) for the leg. Palmitate concentration, palmitate specific activity, CO₂ specific activity and palmitate oxidation rate (Rₚ palmitate) (Fig. 2) did not change significantly throughout exercise. Derived parameters for total 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; 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.

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fatty acids (concentration, $R_{\text{DFFA}}$ and $R_{\text{EFFA}}$) 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% $\dot{M}_{\text{Omax}}$.

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 $R_{\text{DFFA}}$), and use circulatory fatty acids faster (higher $R_{\text{EFFA}}$) 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. 1996a), 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 larger in dogs than in goats. We conclude that the design of the more convoluted pathway involving the use of circulatory fatty acids is lower.

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**References**


