Increased capacity for circulatory fatty acid transport in a highly aerobic mammal

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McClelland, Grant, Georges Zwingelstein, C. Richard Taylor, and Jean-Michel Weber. Increased capacity for circulatory fatty acid transport in a highly aerobic mammal. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35): R1280-R1286, 1994.—Plasma fatty acid (FA) and albumin concentrations, cardiac output, and hematocrit of dogs and goats [dog-to-goat ratio of maximal oxygen consumption ($\dot{V}O_{2max}$) = 2.2] were measured to determine rates of circulatory FA delivery during exercise. Our goals were *1*) to characterize the mechanism(s) used by the endurance-adapted species (dog) to support higher rates of FA delivery to working muscles than the sedentary species (goat) and *2*) to determine whether circulatory transport is scaled with $\dot{V}O_{2max}$. Lipid oxidation was 2.5 times higher in dogs than in goats. Dogs had higher cardiac outputs than goats, but this positive effect on their FA delivery was canceled by higher hematocrit. Dogs always had higher plasma FA concentrations than goats. In contrast, albumin was steady and identical in both species, showing that dogs transport FA at higher rates than goats only because they load more FA on their albumin. Average dog-to-goat ratios for FA delivery (1.5–2.0) were lower than would be expected if this rate were scaled with $\dot{V}O_{2max}$. In vitro experiments showed that dog albumin is designed for high rates of FA transport because it can bind 50% more FA than goat albumin. All endurance-adapted species may possess such "aerobic albumins" to supply more circulating FA to their working muscles than sedentary species.

lipid metabolism; oxidative fuel; free fatty acid; nonesterified fatty acids; plasma proteins; albumin; endurance exercise; cardiac output

MOST VERTEBRATES store enough triacylglycerols in adipose tissue to power moderate exercise for several days (14, 19), and species adapted for endurance locomotion should have an increased capacity to use these extensive energy reserves (26). In mammals, we have shown recently that the processes of fatty acid (FA) mobilization from adipocytes (27) and FA oxidation in locomotory muscles (20) are both scaled with aerobic capacity [mass-specific maximal oxygen consumption ($\dot{V}O_{2max}$)]. At equivalent exercise intensities (same $\%\dot{V}O_{2max}$), highly aerobic dogs are able to support more than twice the lipolytic and FA oxidation rates of sedentary low-aerobic goats (dog-to-goat ratio of $\dot{V}O_{2max}$ = 2.2). These results suggest that circulatory FA transport, the intermediate step between adipose mobilization and muscle oxidation, is similarly scaled with $\dot{V}O_{2max}$. However, this is not necessarily the case, because triacylglycerols stored directly in muscles are also an important source of energy (3, 26), and their relative contribution could increase with aerobic capacity as suggested by endurance training studies (16). Increased supply rates of both circulatory FA and muscle triacylglycerol-derived FA should contribute to the high rates of total lipid oxidation of endurance-adapted mammals.

FA are transported from adipose tissue to active muscles in plasma, but not in red blood cells, and their rate of delivery (μmol/min) can therefore be expressed as

$$\text{FA delivery} = \dot{Q}_{blood} \times (1 - \text{Hct}) \times [\text{FA}]_{plasma} \qquad (1)$$

where $\dot{Q}_{blood}$ is whole blood cardiac output (ml/min), Hct is hematocrit (%), $[FA]_{plasma}$ is fatty acid concentration in plasma (μmol/ml), and $\dot{Q}_{blood} \times (1-\text{Hct})] = \dot{Q}_{plasma}$ or plasma cardiac output (ml/min). Higher hematocrit is an important adaptation for endurance because it increases oxygen delivery (18), but it is clear from *Eq. 1* that it has the opposite effect on FA delivery. Here, endurance adaptation could have occurred at different levels because each parameter of *Eq. 1* could be involved in allowing highly aerobic species to supply more FA to working muscles than sedentary species. In addition, because FA are almost insoluble in aqueous solutions, albumin serves as a "fatty acid carrier" in the same way hemoglobin is an "oxygen carrier." Thus maximal circulatory transport of FA could be limited by the availability (22, 25) and/or the binding capacity of plasma albumin.

The goal of this study was to measure the key parameters determining rates of circulatory FA transport in exercising dogs and goats to establish *1*) the mechanism allowing endurance-adapted species to support higher FA delivery than low-aerobic species and *2*) whether circulatory FA transport is scaled with aerobic capacity. In this context, the role of albumin was also investigated to find out whether changes in the concentration and/or binding capacity of this plasma protein are involved in endurance adaptation.

METHODS

Animals, training, and exercise protocols. For 5 mo before these experiments, three African pigmy goats (*Capra hircus*, 2 females and 1 male, 31.0 ± 1.8 kg) and 3 dogs (*Canis familiaris*, all females, 24.9 ± 1.3 kg) were trained at least four times a week to run on a motorized treadmill at different speeds until their oxygen consumption ($\dot{V}O_2$) was reproducible at each speed. $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) were measured with an open-flow system as described by Fedak et al (7). All the training and experiments were performed at 18 and 29% incline for goats and dogs, respectively. $\dot{V}O_{2max}$ of every animal used in this study had been measured previously (20) ($\dot{V}O_{2max}$ for goats = 1.10 ± 0.04 and for dogs = 2.43 ± 0.05 ml

$O_2 \cdot kg^{-1} \cdot s^{-1}$), and each animal was specifically trained to run for 2 h at 40% $\dot{V}O_{2max}$, 1 h at 60% $\dot{V}O_{2max}$, and 16 min at 85% $\dot{V}O_{2max}$. Measurements were carried out at three intensities because the relative importance of lipid oxidation strongly depends on work rate (8). $\dot{V}O_2$ and $\dot{V}CO_2$ were measured throughout exercise to quantify total lipid oxidation according to the equations of Frayn (10), as described previously (27). In RESULTS, $\dot{V}O_2$ and $\dot{V}CO_2$ were converted to micromoles per kilogram per minute to give $\dot{M}O_2$ and $\dot{M}CO_2$, respectively. Recovery was also monitored because the highest plasma FA concentrations are known to occur shortly after the end of prolonged exercise in some species, including humans (2, 28).

The goats were fed hay and were housed in a large outdoor paddock with shelter. The dogs were fed dry dog chow (25% protein, 10% fat, 5% fiber) and were housed in a large indoor-outdoor run. Food was withheld for 18 h before measurements, but the animals had unlimited access to water.

FA and albumin concentrations. A PE-50 catheter was placed in the aorta via the carotid artery, as described previously (27), and blood samples were drawn before, during, and for 1 h after each exercise protocol. The three exercise intensities were measured in different tests, and two experiments on the same animal were always separated by ≥ 1 wk. Blood samples (2 ml each) were spun, and the plasma was frozen immediately. Total FA concentration was measured on a Hewlett-Packard 5890 series II gas chromatograph equipped with an automatic injection system (HP-7673) and flame ionization detector. FA were extracted and methylated via an HCl-catalyzed reaction according to Tserng et al. (24) with reaction time increased to 20 min. Analyses were performed on a 30-m fused-silica capillary column (DB-23, J & W Scientific, Folsom, CA) kept at 170°C for 35 min after injection, raised to 220°C at a rate of 50°C/min thereafter, and maintained at 220°C until complete column drain (46 min). Injection port and detector temperatures were 210 and 250°C, respectively. The gas chromatograph was calibrated with FA standards (Supelco, Bellefonte, CA), and heptadecanoic acid was used as an internal standard for plasma samples. Albumin concentration was measured with the bromcresol green binding technique at pH 4.0 on a Pye Unicam 8600 spectrophotometer at 600 nm after calibration curves were generated from commercial goat and dog albumin samples (Sigma Chemical, St. Louis, MO). A molecular weight of 66,000 was used to calculate concentrations in nmol/ml. FA binding to albumin was investigated in vitro according to Glatz and Veerkamp (12) with use of [1-^{14}C]oleate (New England Nuclear, Mississauga, Ont.; sp act of 53.5 mCi/mmol) and purified albumin from goat and dog (Sigma Chemical). Albumin samples were delipidated with isopropylether, freeze-dried, and resuspended in different volumes of phosphate-buffered saline (120 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4) to obtain albumin solutions of 0.1 to 1 nmol/ml. Two nmol of [1-^{14}C]oleate in dioxane-propylene glycol (2:1 vol/vol) were incubated in 0.5 ml of each albumin solution. Bound and free FA were separated with Lipidex 1000 (Packard Instruments) (12).

Cardiac output. In separate experiments, arterial oxygen content (Ca_{O_2}), mixed venous oxygen content (Cv_{O_2}), and mass-specific oxygen consumption [$\dot{V}O_2/M_b$, (7)] were measured simultaneously to calculate $\dot{Q}_{blood}$ with the Fick equation. Three measurements were made through each exercise protocol to ensure that $\dot{Q}_{blood}$ was in steady state. No differences were observed between consecutive measurements at the same exercise intensity, and mean values ($n = 3$) have been used for calculations. Catheters (PE-50) were placed in the aorta via the carotid artery and in the pulmonary artery via the jugular vein to sample arterial and mixed venous blood. Their exact position was confirmed by pressure tracings. Hematocrit was calculated from hemoglobin concentration by linear interpolation from previously measured values (23). Hemoglobin was assayed spectrophotometrically (kit 525, Sigma Chemical), and blood oxygen content was measured with a modified Tucker method (23).

Statistics. Results were analyzed by one- or two-way analysis of variance (ANOVA) with replication. For albumin and FA concentrations, Tukey's test was used to compare exercise and recovery values with resting levels. Linear regressions were performed on exercise values for each species separately to determine whether these parameters changed over time. In cases where the slopes were significant in both species, an analysis of covariance (ANCOVA) was used to test whether they were different from each other. All values presented are means ± SE.

RESULTS

Indirect calorimetry. Oxygen consumption ($\dot{M}O_2$), carbon dioxide production ($\dot{M}CO_2$), respiratory exchange ratios, and calculated rates of total lipid oxidation for both species throughout exercise are presented in Table 1. Total lipid oxidation, $\dot{M}O_2$, and $\dot{M}CO_2$ were significantly higher in dogs than in goats at each exercise intensity ($P < 0.01$), and the ratio between dogs and goats was higher than 2. The respiratory exchange ratio

Table 1. *Oxygen consumption, CO_2 production, respiratory exchange ratio, and total lipid oxidation in trained dogs and goats running on a treadmill*

	40% $\dot{V}O_{2max}$			60% $\dot{V}O_{2max}$			85% $\dot{V}O_{2max}$		
	30 min	60 min	90 min	15 min	30 min	45 min	5 min	10 min	15 min
$\dot{M}O_2$, μmol $O_2 \cdot kg^{-1} \cdot min^{-1}$									
Dog	2,454 ± 88	2,410 ± 62	2,383 ± 127	3,516 ± 263	3,713 ± 221	3,927 ± 170	5,257 ± 438	5,489 ± 313	5,741 ± 286
Goat	1,141 ± 67	1,188 ± 70	1,210 ± 38	1,622 ± 88	1,683 ± 59	1,710 ± 65	2,420 ± 218	2,553 ± 236	2,598 ± 224
$\dot{M}CO_2$, μmol $CO_2 \cdot kg^{-1} \cdot min^{-1}$									
Dog	1,938 ± 132	1,870 ± 78	1,787 ± 111	3,121 ± 159	3,302 ± 314	3,393 ± 193	4,710 ± 516	5,119 ± 388	5,397 ± 347
Goat	909 ± 65	907 ± 33	941 ± 68	1,507 ± 90	1,484 ± 50	1,481 ± 44	2,278 ± 256	2,380 ± 257	2,471 ± 257
RER									
Dog	0.79 ± 0.03	0.78 ± 0.02	0.75 ± 0.03	0.89 ± 0.02	0.89 ± 0.04	0.86 ± 0.02	0.89 ± 0.03	0.93 ± 0.02	0.94 ± 0.02
Goat	0.80 ± 0.04	0.77 ± 0.03	0.78 ± 0.03	0.93 ± 0.01	0.88 ± 0.02	0.87 ± 0.02	0.94 ± 0.02	0.93 ± 0.02	0.95 ± 0.02
Lipid oxidation, μmol $O_2 \cdot kg^{-1} \cdot min^{-1}$									
Dog	1,750 ± 175	1,832 ± 120	2,019 ± 218	1,340 ± 352	1,392 ± 385	1,810 ± 210	1,853 ± 274	1,252 ± 291	1,163 ± 392
Goat	788 ± 142	953 ± 182	912 ± 110	390 ± 62	675 ± 108	777 ± 151	483 ± 140	587 ± 132	432 ± 167

Values are means ± SE of 3 dogs and 3 goats. $\dot{V}O_{2max}$, maximal O_2 consumption. $\dot{M}O_2$ and $\dot{M}CO_2$, O_2 consumption and CO_2 production, respectively, converted to μmol $\cdot kg^{-1} \cdot min^{-1}$. RER, respiratory exchange ratios.

was the same in both species ($P > 0.05$), and it increased with exercise intensity. Average values for dogs and goats were 0.77, 0.87, and 0.93 at 40, 60, and 85% $\dot{V}O_{2max}$, respectively.

Albumin. During exercise at all intensities, plasma albumin concentration remained at resting levels, and the two species did not differ from each other ($P > 0.05$). Dogs and goats had an average albumin concentration of 0.54 mM (Figs. 1–3). No change was observed during recovery except for a slight increase at 5 and 10 min after the 60% $\dot{V}O_{2max}$ run in dogs ($P < 0.05$, Fig. 2).

FA. Plasma FA concentration was always higher in dogs than in goats before, during, and after exercise at all intensities ($P < 0.0001$, Figs. 1–3). The difference in concentration between the two species varied during exercise and recovery but was particularly pronounced in early recovery from the 40 and 60% $\dot{V}O_{2max}$ runs. Exercising at 40% $\dot{V}O_{2max}$ caused a steady FA increase in dogs ($P < 0.05$) but had no effect in goats ($P > 0.05$; Fig. 1). A clear FA overshoot was observed in dogs during recovery, when concentration reached a maximum of 3.43 mM at 10 min postexercise. In this species, FA concentration remained significantly higher than at rest for 30 min after the end of the run. In contrast, FA concentration of goats did not change from resting levels troughout exercise or recovery, and no overshoot was observed (Fig. 1). Running at 60% $\dot{V}O_{2max}$ caused an increase in FA concentration for both species (slopes different from 0, $P < 0.02$), but the rate of increase was much higher for dogs than for goats ($P < 0.01$; Fig. 2). Early in recovery, a large overshoot occurred in dogs when a maximum of 3.53 mM FA was observed 5 min postexercise. In this species, concentration returned to resting levels 20 min after the end of the run. No such overshoot was seen in goats, the recovery values of which were never different from rest ($P > 0.05$). During and after the 85% $\dot{V}O_{2max}$ run, FA concentration was never significantly different from resting levels in either species ($P > 0.05$; Fig. 3).

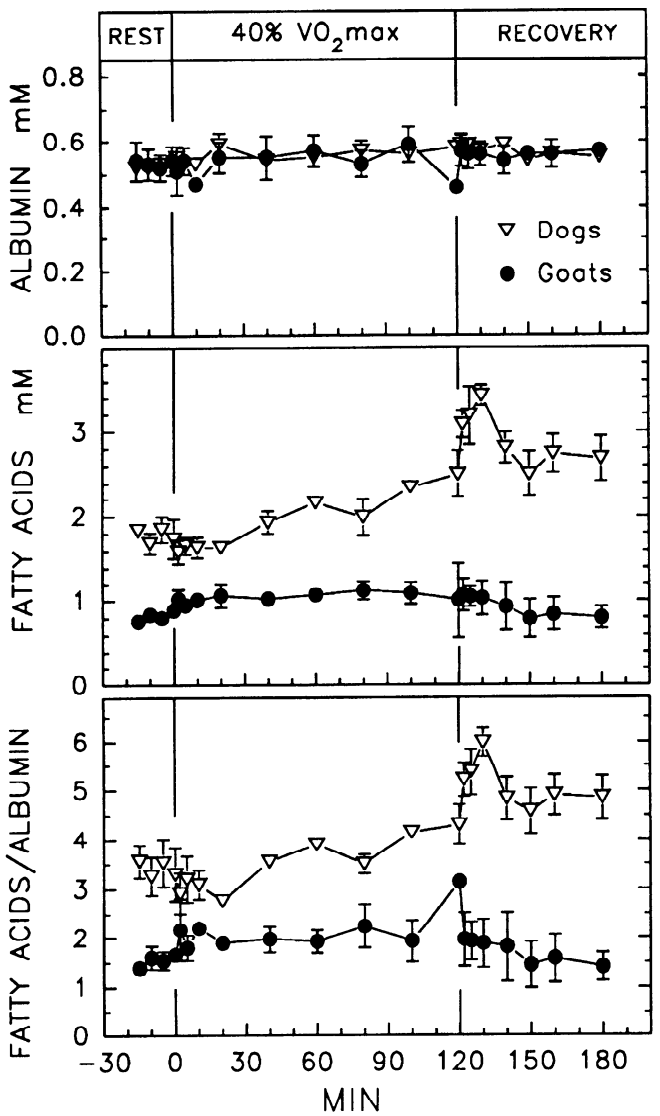

Fig. 1. Albumin concn, total fatty acid concn, and fatty acid-to-albumin ratio in plasma of 3 dogs and 3 African pigmy goats before, during, and after 2 h of treadmill exercise at 40% maximal oxygen consumption ($\dot{V}O_{2max}$).

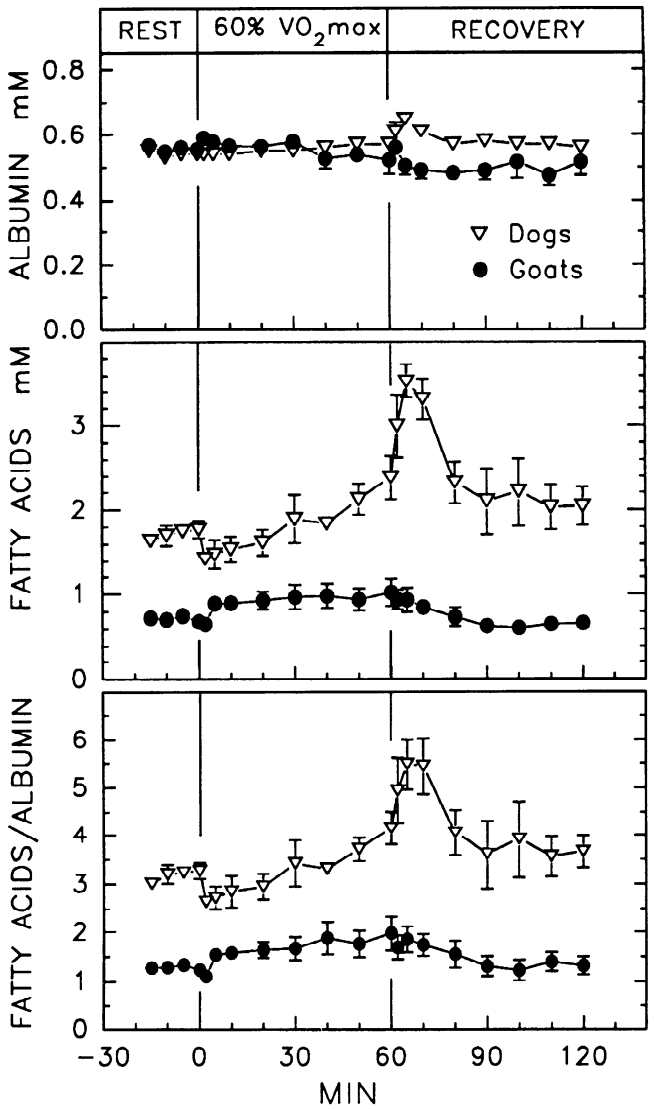

Fig. 2. Albumin concn, total fatty acid concn, and fatty acid-to-albumin ratio in plasma of same animals as in Fig. 1 before, during, and after 1 h of treadmill exercise at 60% $\dot{V}O_{2max}$.

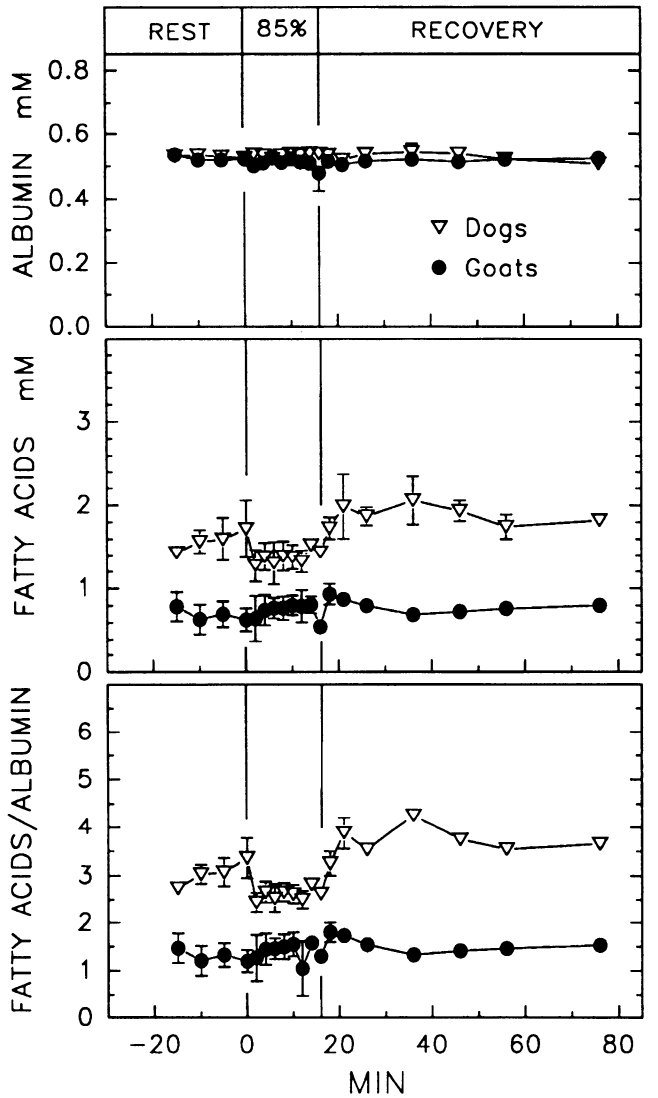

Fig. 3. Albumin concn, total fatty acid concn, and fatty acid-to-albumin ratio in plasma of same animals as in Fig. 1 before, during, and after 16 min of treadmill exercise at 85% $\dot{V}O_{2max}$.

In vivo FA-to-albumin ratio. The FA-to-albumin ratio was much higher in dogs than goats before, during, and after exercise at all intensities ($P < 0.0001$, Figs. 1–3). Overall, the effects of exercise on the number of FA bound per albumin were identical to what was described above for FA alone. During exercise at 40 and 60% $\dot{V}O_{2max}$, a steady increase was observed in dogs but not in goats. During and after the 85% $\dot{V}O_{2max}$ run, the ratio remained at resting values in both species ($P > 0.05$; Fig. 3). The highest values were reached at the end of exercise for goats and in the first 10 min of recovery for dogs. The postexercise FA overshoot of dogs brought this ratio to the maximal in vivo values recorded in these experiments: 5.99 and 5.48 after the 40 and 60% $\dot{V}O_{2max}$ runs, respectively (Figs. 1 and 2).

Cardiac output. Arteriovenous difference in oxygen content ($Ca_{O_2} - Cv_{O_2}$), mass-specific $\dot{V}O_2$ ($\dot{V}O_2/M_b$), hematocrit, and cardiac output for whole blood ($\dot{Q}_{blood}$) and for plasma ($\dot{Q}_{plasma}$) are presented in Table 2. $Ca_{O_2} - Cv_{O_2}$ was always much higher in dogs than in goats ($P <$ 0.001). The measured $\dot{V}O_2$ was 2.1–2.3 times higher in dogs than in goats and represented 40 and 35% $\dot{V}O_{2max}$ for dogs and goats, respectively, at target 40% $\dot{V}O_{2max}$, 54 and 57% at target 60% $\dot{V}O_{2max}$, and 83 and 86% at target 85% $\dot{V}O_{2max}$. For each exercise intensity, dogs had higher hematocrits and higher $\dot{Q}_{blood}$ than goats, but $\dot{Q}_{plasma}$ was the same in both species ($P > 0.05$).

Table 2. *Measured arteriovenous difference in oxygen content, mass-specific oxygen consumption, hematocrit, and calculated cardiac output for whole blood and plasma*

	$Ca_{O_2} - Cv_{O_2}$, ml O_2/dl	$\dot{V}O_2/M_b$, ml $O_2 \cdot kg^{-1} \cdot s^{-1}$	$\dot{Q}_{blood}$ ml $\cdot kg^{-1} \cdot s^{-1}$	Hct, %	$\dot{Q}_{plasma}$ ml $\cdot kg^{-1} \cdot s^{-1}$
40% $\dot{V}O_{2max}$					
Dog	13.1 ± 0.8	0.98 ± 0.01	7.6 ± 0.5	43 ± 3	4.3 ± 0.2
Goat	6.9 ± 0.7	0.38 ± 0.02	5.6 ± 0.7	25 ± 2	4.2 ± 0.6
Dog/goat	1.9	2.6	1.4	1.7	1.0
60% $\dot{V}O_{2max}$					
Dog	15.8 ± 0.3	1.31 ± 0.03	8.3 ± 0.3	45 ± 2	4.6 ± 0.1
Goat	8.2 ± 0.7	0.63 ± 0.07	7.9 ± 1.5	27 ± 1	5.8 ± 1.2
Dog/goat	1.9	2.1	1.1	1.7	0.8
85% $\dot{V}O_{2max}$					
Dog	15.9 ± 0.2	2.01 ± 0.07	12.6 ± 0.4	48 ± 3	6.5 ± 0.4
Goat	10.5 ± 0.4	0.95 ± 0.07	9.1 ± 0.6	29 ± 2	6.4 ± 0.7
Dog/goat	1.5	2.1	1.4	1.7	1.0

Measurements (means ± SE) were performed in dogs ($n = 3$) and African pigmy goats ($n = 3$) running at target exercise intensities of 40, 60, and 85% $\dot{V}O_{2max}$. $Ca_{O_2} - Cv_{O_2}$, arteriovenous difference in oxygen content; $\dot{V}O_2/M_b$, mass-specific oxygen consumption; Hct, hematocrit; $\dot{Q}_{blood}$ and $\dot{Q}_{plasma}$, calculated cardiac output for whole blood and plasma, respectively.

Circulatory FA delivery. The rate of FA delivery was higher in dogs than in goats at all exercise intensities ($P < 0.0001$; Figs. 4–6). In dogs, it increased significantly throughout exercise at 40 and 60% $\dot{V}O_{2max}$ ($P < 0.01$). No significant change in delivery rate was measured during exercise at any intensity for goats or at

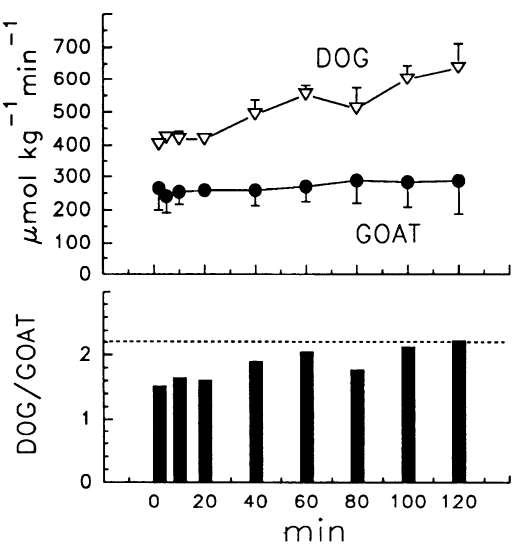

Fig. 4. Circulatory fatty acid delivery in 3 dogs and 3 goats (*top*) and ratio between dogs and goats (*bottom*) during exercise at 40% $\dot{V}O_{2max}$. Dashed line indicates a dog-to-goat $\dot{V}O_{2max}$ ratio of 2.2 (see METHODS).

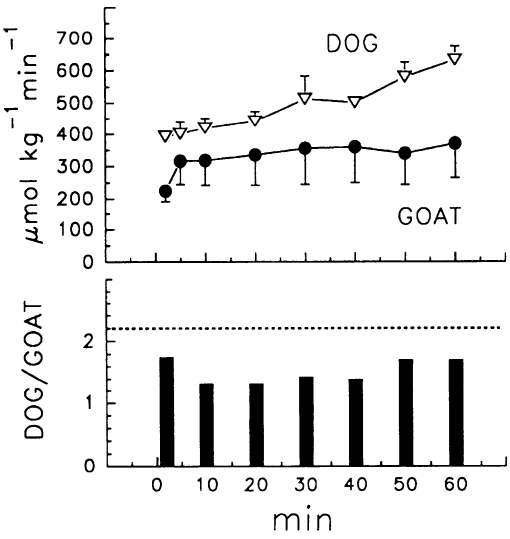

Fig. 5. Circulatory fatty acid delivery and ratio between dogs and goats during exercise at 60% $\dot{V}O_{2max}$. Dashed line indicates a dog-to-goat $\dot{V}O_{2max}$ ratio of 2.2.

85% $\dot{V}O_{2max}$ for dogs ($P > 0.5$). The ratio between FA delivery in dogs and in goats reached the value of 2.2 (dog/goat $\dot{V}O_{2max}$ ratio represented by the dashed lines in Figs. 4–6) during the 40 and 85% $\dot{V}O_{2max}$ runs but only attained a maximum of 1.75 in the 60% $\dot{V}O_{2max}$ run. Average dog-to-goat ratios throughout exercise were 1.86, 1.52, and 1.98 at 40, 60, and 85% $\dot{V}O_{2max}$, respectively.

In vitro FA-albumin binding. Dog albumin was able to bind significantly more FA than goat albumin ($P < 0.0001$). At the lowest albumin concentration tested in vitro (0.1 nM), each albumin molecule could bind 4.55 ± 0.56 FA for goats and 6.75 ± 0.45 for dogs (Fig. 7). These were the highest ratios measured in this study for each species.

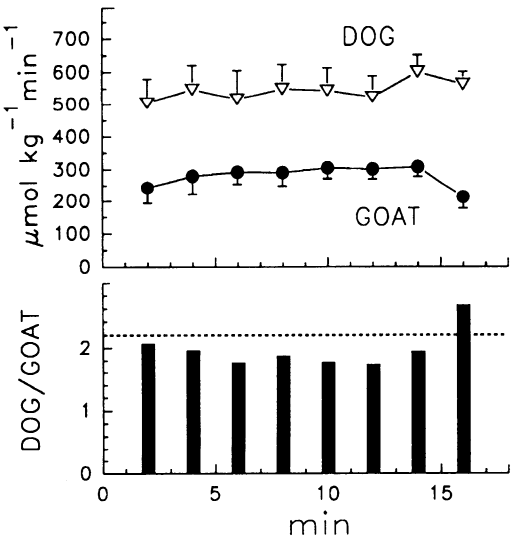

Fig. 6. Circulatory fatty acid delivery and ratio between dogs and goats during exercise at 85% $\dot{V}O_{2max}$. Dashed line indicates a dog-to-goat $\dot{V}O_{2max}$ ratio of 2.2.

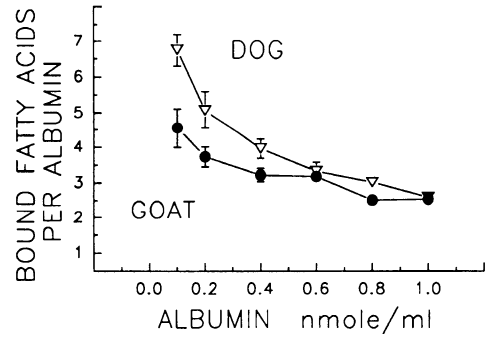

Fig. 7. In vitro fatty acid binding to dog and goat albumin. Values are ratios (means ± SD; $n = 4$) of [1-^{14}C]oleate bound to albumin vs. albumin concn. Same amount of [1-^{14}C]oleate was incubated with solutions of decreasing albumin concn (see METHODS).

DISCUSSION

Our results show that endurance-adapted dogs transport circulatory FA at much higher rates than low-aerobic goats because they load more FA on their plasma albumin (Figs. 1–3). This adaptation alone is responsible for the remarkable difference in transport capacity between the two species, because other strategies, such as increasing plasma flow (Table 2) or increasing albumin concentration (Figs. 1–3), are not used by dogs to enhance FA supply to their working muscles. Dogs oxidize lipids at more than twice the rate of goats (Table 1), and their high circulatory FA transport contributes to this increased capacity for lipid use during locomotion. Total lipid oxidation decreased as exercise intensity increased (Table 1), and these results may be explained by lactate inhibition at the highest work rates (17).

Hematocrit and cardiac output. At each exercise intensity, both species show identical plasma flows ($\dot{Q}_{plasma}$) because the higher $\dot{Q}_{blood}$ and higher hematocrit of dogs have opposite and canceling effects. Although a high hematocrit plays a major role in allowing dogs to transport 2.2 times more oxygen than goats (18), it undoubtedly impairs their FA delivery. Evolutionary changes in hematocrit to improve endurance capacity have clearly favored the transport of oxygen over FA. This trade-off may have been realized because oxygen cannot be stored significantly in locomotory muscles whereas FA can. Nevertheless, the negative effect of high hematocrit on the FA delivery of dogs is exactly compensated by another structural adaptation of their circulatory system: their larger hearts allow them to increase stroke volume (18) and consequently $\dot{Q}_{blood}$, thereby achieving the same $\dot{Q}_{plasma}$ as goats (Table 2).

Plasma albumin levels. Endurance-adapted dogs do not use higher plasma albumin concentrations than sedentary goats to increase FA delivery. Albumin was maintained between 0.5 and 0.6 mM in both species and at all work rates (Figs. 1–3). Other mammals have similar plasma levels, and studies on humans show that exercise does not affect concentration (5) or causes only a weak increase (9, 15). Such stability implies that albumin levels in plasma are probably constrained by functional demands unrelated to lipid metabolism. This compound makes up 50–60% of total plasma proteins,

and it is responsible for 80% of the colloid osmotic pressure (1, 13). Therefore, the major role it plays in osmoregulation provides the most likely explanation for the stability of its concentration.

Relationship between FA delivery and $\dot{V}O_{2max}$. Overall, our results show that circulatory FA transport during exercise is not exactly scaled with aerobic capacity (Figs. 4–6), even though the dog-to-goat delivery rate ratio reaches the value of 2.2 at the very end of the 40 and 85% $\dot{V}O_{2max}$ runs. Averaged throughout exercise, the dog-to-goat ratio was only 1.86, 1.52, and 1.98 at the three work intensities, and the highest FA transport rates measured in this study (i.e., 639 μmol $FA \cdot kg^{-1} \cdot min^{-1}$ for dog and 370 μmol $FA \cdot kg^{-1} \cdot min^{-1}$ for goat) yield a ratio of only 1.72. Therefore, the delivery rate of the endurance-adapted species is somewhat lower than would be expected if circulatory FA transport were scaled with $\dot{V}O_{2max}$. This observation allows us to infer two additional differences between the two species. First, it indicates that a larger fraction of total FA released from adipose stores undergoes reesterification (see Ref. 28) in dogs than in goats because lipolytic rate is scaled with aerobic capacity (27). Second, because total FA oxidation is also scaled with $\dot{V}O_{2max}$ (Table 1; Ref. 20) but circulatory transport is not, the relative contribution of muscle FA stores to total lipid utilization must be higher in dogs than in goats to make up for the shortfall in convective FA supply. This observation is supported by measurements of muscle triacylglycerol oxidation showing that endurance-trained humans rely proportionately more than untrained individuals on intramuscular stores (16). Also, recent work on the same individual dogs and goats shows that circulatory glucose supply, like plasma FA supply, fails to scale with aerobic capacity (J.-M. Weber, T. J. Roberts, and C. R. Taylor, unpublished observations). General analyses of oxidative fuel provision suggest that circulatory supply cannot be increased beyond a maximum limit (6). The respective transmembrane carrier systems for FA and glucose could be responsible for setting maximal rates of circulatory supply to muscle cells for both substrates (25). Thus highly aerobic animals may rely more than sedentary animals on intramuscular fuel stores to avoid this limitation (25, 26).

The highest delivery rates achieved by dogs at the different exercise intensities are very similar (600–640 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), suggesting that maximum transport is stimulated at all work rates in this species. In contrast, goats showed their maximal delivery at 60% $\dot{V}O_{2max}$, and this observation is consistent with our previous measurement of maximum adipose tissue lipolysis occurring at this same intensity (27).

FA-to-albumin binding. Both our in vivo and in vitro measurements of FA-to-albumin ratios show that dog albumin binds significantly more FA than goat albumin (Figs. 1–3 and 7). In whole animal experiments, the number of FA bound to albumin was always much higher in dogs than in goats, and a twofold difference between species was reached toward the end of the low- and medium-intensity exercise bouts. This difference in FA loading was even more obvious during recovery, when dogs showed a large overshoot in FA concentration, thereby bringing their FA-to-albumin ratio to a maximum of 6:1, or over three times the highest recovery value of goats (Figs. 1 and 2). Maximal ratios between 5 and 7 have also been reported in vitro for other mammalian albumins (4, 21) and in electrically stimulated rat muscle (11) and in vivo for exercising humans (15, 28). Unfortunately, the wide variety of experimental approaches and animal sizes used in these other studies prevents the extension of our observed correlation between maximal albumin loading and aerobic capacity to mammals in general.

Our in vivo measurements of FA loading do not allow us to determine whether the maximal binding capacity of dog and goat albumin is intrinsically different. Indeed, goats, and possibly dogs, could simply not use their maximal binding capacity in vivo, and this issue prompted our in vitro experiments. Results clearly show that the intrinsic binding capacity is much higher in the endurance-adapted than in the sedentary species, with dogs displaying a maximal in vitro ratio 50% greater than goats (Fig. 7).

Recovery and control of lipolysis. The large overshoot in FA concentration seen in dogs immediately after exercise (Figs. 1 and 2) shows that this species, like humans, probably lacks rapid control mechanisms for lipolysis (2, 27, 28). It has been suggested that high plasma FA concentrations in recovery could inhibit muscle glycolysis through the glucose-FA cycle (2), thereby increasing glucose availability for muscle glycogen replenishment. Unlike dogs and humans, goats cannot use this mechanism to regulate postexercise fuel oxidation because they show no overshoot in FA concentration (Fig. 1–3). However, this absence of overshoot and their ability to return lipolytic rate to resting values immediately after the end of exercise (27) suggest that the control mechanisms for lipid mobilization are much more rapid in goats than in dogs and humans.

In conclusion, we show that highly aerobic mammals like dogs possess an "aerobic albumin" with elevated FA binding capacity. This adaptation allows them to support much higher rates of circulatory FA transport than sedentary goats, but further research is needed to determine whether all endurance-adapted species have evolved such albumin. We suggest that albumin-FA binding capacity is correlated with $\dot{V}O_{2max}$.

We thank Scott Graham and Chris Saucedo for their great help in measuring cardiac output and Tim West for commenting on an early draft of the manuscript.

During this study, G. McClelland was supported by a grant from the French Ministry of External Affairs; his present address: Dept. of Zoology, University of British Columbia, Vancouver, BC V6T 2A9, Canada.

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Received 10 February 1993; accepted in final form 13 October 1993.

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