The Journal of Experimental Biology **199**, 1659–1666 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0188

DESIGN OF THE OXYGEN AND SUBSTRATE PATHWAYS

III. PARTITIONING ENERGY PROVISION FROM CARBOHYDRATES

JEAN-MICHEL WEBER[1,2,*], THOMAS J. ROBERTS[1], RUTH VOCK[3], EWALD R. WEIBEL[3] AND C. RICHARD TAYLOR[1]

[1]*Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA,* [2]*Biology Department, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5* and [3]*Department of Anatomy, University of Berne, CH-3000 Berne, Switzerland*

Accepted 3 April 1996

Summary

This paper quantifies maximal fluxes through the pathway supplying carbohydrates to the mitochondria of muscle cells. Continuous infusions of D-[3-^{3}H]glucose together with indirect calorimetry were used to investigate the partitioning of the supply of carbohydrates through the two branches of the pathway: from circulating glucose and from glycogen stores within the muscle cells to the mitochondria. The relative contribution of circulating glucose to total carbohydrate oxidation was small, accounting for only 13 % and 23 % of the carbohydrate oxidized at exercise intensities approaching $\dot{M}_{O_2max}$ in dogs and goats, respectively. Unexpectedly, maximal rates of circulating glucose oxidation were nearly the same in the two species (when expressed in absolute terms; dog:goat ratio = 1.2), despite the 2.2-fold difference in aerobic capacity. We conclude that the glycogen stores in the muscle cells are the major source of substrates at maximal rates of oxidation, supplying 60–70 % of the total energy. Furthermore, it is this branch of the carbohydrate pathway that is adapted to the large difference in aerobic capacity between dogs and goats.

Key words: glucose oxidation, muscle glycogen, exercise, metabolism, symmorphosis, dog, goat.

Introduction

In the preceding paper of this series (Roberts *et al.* 1996), we have quantified the contributions of carbohydrate and lipid oxidation to total aerobic metabolism in running dogs and goats. We have shown that maximal rates of lipid oxidation are reached at low exercise intensity and that the extra energy required at higher work rates is provided exclusively by carbohydrate oxidation. Furthermore, we found that dogs and goats show the same pattern of fuel utilization, but that the rates of lipid and carbohydrate oxidation are proportional to the 2.2-fold difference in aerobic capacity between the athletic and the sedentary species.

In this paper, we focus our attention on carbohydrate supply to the mitochondria of exercising muscles. The carbohydrate pathway to working muscle mitochondria has two branches (one delivering glucose from the circulation, the other delivering glucosyl units from nearby glycogen stores within the muscle cells) and these two branches involve different structural elements (Taylor *et al.* 1996). This is shown in our model of Fig. 1, where step 1 represents the rate of glucose entry into the circulation from the liver and gut, $\dot{M}_{CHO}$(in), which corresponds to R_a glucose, the abbreviation commonly used in metabolic biochemistry. Similarly, step 2 represents the rate of disappearance of glucose from the circulation, or R_d glucose. Under the exercise conditions of our experiments, R_d glucose is a good approximation of the rate at which circulating glucose is being oxidized, $\dot{M}_{CHO}$(iv). This approximation is justified because several studies in dogs and humans, where rates of glucose disappearance and oxidation were measured simultaneously, show that more than 90 % of R_d glucose is accounted for by glucose oxidation during low-intensity exercise (Coggan *et al.* 1990; Paul and Issekutz, 1967). Our calculations could thus lead to an overestimation of the absolute rate of glucose oxidation by up to 10 %, but this potential error is likely to become negligible at high exercise intensities when the rate of non-oxidative glucose disposal approaches zero. Step 3 of our model is the rate of oxidation of glucosyl units supplied from glycogen stores in muscle cells, $\dot{M}_{CHO}$(ic). Finally, step 4 is the total rate of mitochondrial glucose oxidation from both branches of the pathway, $\dot{M}_{CHO}$(mt). It corresponds to the summed contributions of circulating glucose and intramuscular glycogen:

$$\dot{M}_{CHO}(mt) = \dot{M}_{CHO}(iv) + \dot{M}_{CHO}(ic) .$$

*Author for correspondence at address 2.

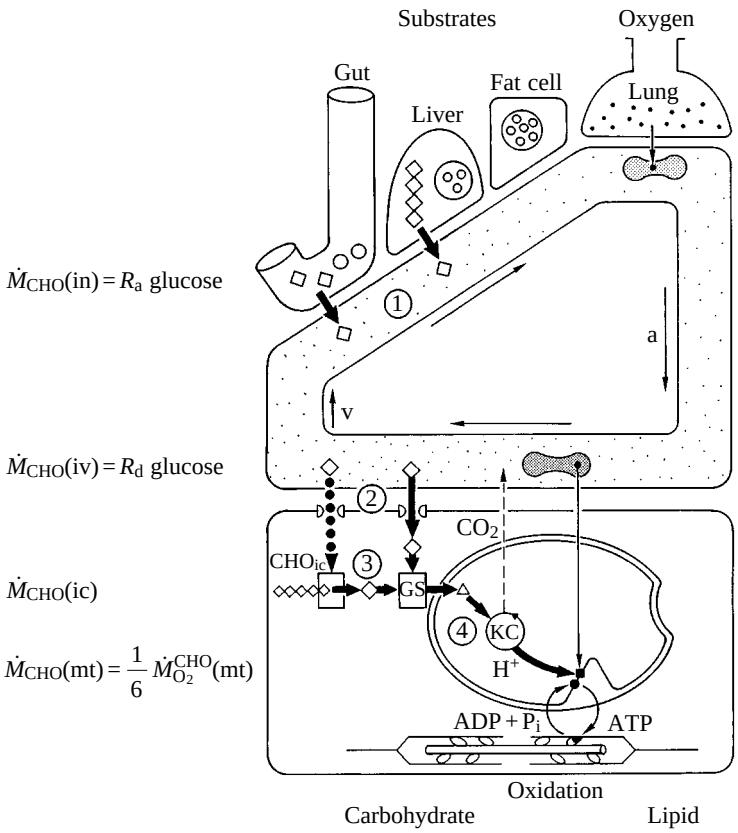

Fig. 1. Model of the carbohydrate pathway indicating (heavy arrows) the glucose supply (squares) to locomotory muscle mitochondria from the circulation and from intramuscular glycogen stores. Note that the supply of glucose to glycogen (dotted arrows) is temporally split from the oxidation of glucose. The O_2 pathway is marked with dots and thin arrows, CO_2 discharge with broken arrows. The triangle marks acetyl-CoA. a, arterial; v, venous; in, 'inflow'; iv, intravascular; ic, intracellular (intramuscular); mt, mitochondrial; CHO_{ic}, intramuscular glycogen stores; GS, glycolysis; KC, Krebs cycle; H^+, reducing equivalents; black square, terminal oxidase in the inner mitochondrial membrane. Step 1, rate of appearance of glucose in the circulation; R_a glucose=$\dot{M}_{CHO}$(in). Step 2, rate of disappearance of glucose from the circulation; R_d glucose=$\dot{M}_{CHO}$(iv), divided into direct (heavy arrow) and indirect (dotted arrow) routes. Step 3, rate of glucose supply from muscle glycogen stores; $\dot{M}_{CHO}$(ic). Step 4, rate of total carbohydrate utilization in mitochondria; $\dot{M}_{CHO}(mt)=\dot{M}_{CHO}(iv)+\dot{M}_{CHO}(ic)$, which equals one-sixth of O_2 consumption induced by glucose $\dot{M}_{O_2}^{CHO}$(mt).

Here, we investigate the partitioning of the energy supply between the two branches of the carbohydrate pathway in order to provide the functional data needed to test the principle of symmorphosis by correlating maximal rates through each branch of the pathway with the corresponding structural parameters determining the capacity of the different transport steps.

Materials and methods

The four pygmy goats and three Labrador dogs used for this study were the same animals as those used in the previous paper (Roberts *et al.* 1996), where surgical procedures, training schedule, measurement of $\dot{M}_{O_2max}$ and exercise protocols are described.

Catheterization

The day before measuring glucose kinetics, a sterile PE-50 catheter was fed through the jugular vein into the pulmonary artery under local lidocaine anaesthesia for infusion of labelled glucose. 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 in the aorta *via* the carotid artery previously moved to a subcutaneous position (Roberts *et al.* 1996). 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. Venous tracer infusion and arterial blood sampling have been used extensively for *in vivo* metabolic studies in rats (Turcotte *et al.* 1990; Vissing *et al.* 1988), dogs (Paul and Issekutz, 1967; Moates *et al.* 1988) and humans (Friedman *et al.* 1991; Katz *et al.* 1992; Molina *et al.* 1990; Weber *et al.* 1990) because this technique provides the most accurate measurement of glucose kinetics (Katz, 1992). It has also been demonstrated that the error introduced by using different, non-optimal infusion/sampling sites is very small in the case of glucose (Jahoor *et al.* 1988; Katz, 1992; Raman *et al.* 1990).

Labelled glucose infusions

Animals were fed each day after they exercised on the treadmill. Thus, they had not eaten for about 24 h before the infusion experiments. All the measurements were made while animals ran up an inclined treadmill (18 % incline for goats and 29 % for dogs) and ambient temperature was maintained between 6 and 14 °C. Experiments were limited to the hours between 08:00 and 15:00 h to minimize any effects of circadian rhythms. A continuous infusion of D-[3-^{3}H]glucose was started while the animal was resting on the treadmill using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA). The use of glucose tritiated in position 3 has been validated thoroughly and it is infused routinely to quantify glucose kinetics (Katz *et al.* 1992; Levy *et al.* 1989; Molina *et al.* 1990). A priming dose equivalent to 90 min of resting infusion was injected as a bolus before starting the pump. Infusions were initiated 1 h before exercise to allow the labelled glucose

to reach isotopic steady state before measuring resting glucose kinetics. They were continued for 1 h after the exercise was finished to monitor recovery. The animals were trained to stand quietly on the treadmill during the pre- and post-exercise resting periods.

The radiolabelled glucose had a specific activity of 289 GBq mmol^{-1} and was purchased from Amersham (Arlington Heights, IL, USA). The exact infusion rate was determined by counting a sample of the infusate. Infusion rates at rest and during recovery averaged 19 888±399 disints min^{-1} kg^{-1} min^{-1} for the goats in all of the experiments (N=12), but they varied somewhat in the dogs, being: 36 645±2241 disints min^{-1} kg^{-1} min^{-1} (N=3) in the experiments at 40 %, 25 504±1013 disints min^{-1} kg^{-1} min^{-1} (N=3) at 60 % and 94 888±1011 disints min^{-1} kg^{-1} min^{-1} (N=3) at 85 % $\dot{M}_{O_2max}$. In both species, infusion rates were increased above resting rates by a factor of 1.8 at an exercise intensity of 40 % $\dot{M}_{O_2max}$ and 2.4 at 60 % and 85 % $\dot{M}_{O_2max}$. These changes in infusion rate were selected to reduce fluctuations in glucose specific activity (Levy *et al.* 1989; Molina *et al.* 1990; Weber *et al.* 1990, 1993), thereby minimizing errors caused by the use of the non-steady-state single-pool model of Steele (1959). 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 residual activity from the previous experiment was present exclusively in water. Animals wore a loose-fitting mask throughout the infusions that allowed the continuous measurement of the rates of O_2 consumption ($\dot{M}_{O_2}$) and CO_2 production ($\dot{M}_{CO_2}$) as described previously (Roberts *et al.* 1996).

Blood sampling

During the glucose infusions, a series of 1.5 ml blood samples was collected from the catheterized aorta into Eppendorf tubes. Plasma was immediately separated by centrifugation and frozen until further processing. Samples were drawn before the exercise period at 45, 50, 55 and 60 min after the start of infusion; after 2, 5, 10, 20, 40, 60, 80, 100 and 120 min of exercise during the 40 % $\dot{M}_{O_2max}$ runs; after 2, 5, 10, 20, 30, 40, 50 and 60 min of exercise during the 60 % $\dot{M}_{O_2max}$ runs; every 2 min during the 85 % $\dot{M}_{O_2max}$ runs; and 2, 5, 10, 20, 30, 40, 50 and 60 min after the end of all exercise bouts.

Analyses

Plasma glucose and lactate concentrations were measured at 340 nm on a Beckman 24 spectrophotometer (Beckman Instruments, Irvine, CA, USA) using standard enzymatic methods (Bergmeyer, 1974). Glucose radioactivity was measured after removing water in a drying oven at 60 °C (Levy *et al.* 1989), resuspending the residue in distilled water, and mixing with ACS II scintillation fluid (Amersham, Arlington Heights, IL, USA). This procedure was shown to be adequate for isolating activity in glucose because plasma radioactivity is present exclusively in glucose and water (Katz *et al.* 1974). Counting was performed on a Beckman LS 1801 scintillation counter with external quench correction. Tritium counting efficiency ranged from 38 to 43 %.

Calculations and statistics

Rates of glucose appearance [R_a glucose=$\dot{M}_{CHO}$(in)] and glucose disappearance [R_d glucose=$\dot{M}_{CHO}$(iv)] were calculated using the steady-state (rest) and non-steady-state equations of Steele (exercise and recovery) (Steele, 1959). The effective volume of distribution for glucose was assumed to be 100 ml kg^{-1} (Weber *et al.* 1990), but increasing isotope infusion rates during exercise as described above minimized the impact of this assumption on the non-steady-state calculations (Levy *et al.* 1989; Weber *et al.* 1990; Wolfe *et al.* 1990). Rates of total carbohydrate and of total lipid oxidation were calculated from the ratio of carbon dioxide produced to oxygen consumed as described previously (Roberts *et al.* 1996). Oxidation rates of intramusclar glycogen stores were estimated by subtracting the contribution of circulating glucose [$\dot{M}_{CHO}$(iv)] from total carbohydrate oxidation [$\dot{M}_{CHO}$(mt)].

Results for dogs and goats were compared using two-way analyses of variance (ANOVAs) with replication, after arcsine transformation when the results were percentages. Within each species, mean values measured during and after exercise were compared with resting levels using a two-way Dunnett's multiple-range test (Zar, 1984). All values presented in the paper are means and standard errors.

Results

Glucose concentration and specific activity

The plasma glucose concentration of dogs averaged 5.4 mmol l^{-1} throughout the experiments (Figs 2–4, top panels) and did not change significantly between rest, exercise at any intensity and recovery (P>0.05). In contrast, plasma glucose concentration of goats increased during exercise and remained elevated well into the recovery period. The resting values were 4.4 mmol l^{-1}, increasing to 9.4 mmol l^{-1} at 40 % and 60 % $\dot{M}_{O_2max}$ and 11.5 mmol l^{-1} at 85 % $\dot{M}_{O_2max}$. All exercise values beyond 20 min of work (40 % $\dot{M}_{O_2max}$) and beyond 5 min (60 and 85 % $\dot{M}_{O_2max}$) were significantly higher than resting values (P<0.01). All values during the recovery period remained above pre-exercise levels after the 60 and 85 % $\dot{M}_{O_2max}$ runs (P<0.01) (Figs 3, 4). 1 h of recovery was just sufficient for glucose concentration to return to resting levels after the 40 % $\dot{M}_{O_2max}$ run (Fig. 2). Plasma lactate concentrations during the experiments are presented in Table 1 and in the top panels of Figs 2–4.

Isotopic steady state was reached during the resting periods, as indicated by the nearly constant specific activity of glucose. Increasing the rate of isotope infusion during exercise and returning this rate to pre-exercise values during recovery minimized the changes in specific activity during the experiments (middle panel of Figs 2–4).

Rate of appearance of glucose in the circulation

Glucose entered the circulation at higher rates in dogs than

in goats at 40 and 60 % $\dot{M}_{O_2max}$ (P<0.0001, Figs 2, 3), but there was no difference between the species at the highest exercise intensity of 85 % $\dot{M}_{O_2max}$ (Fig. 4, P=0.17). Overall, R_a glucose changed significantly over time in both species and at all exercise intensities (ANOVA, P<0.0001). In dogs, exercise values of R_a glucose were higher than rest values after 30, 10 and 8 min of exercise at 40, 60 and 85 % $\dot{M}_{O_2max}$, respectively (P<0.01), but stayed elevated for 20 and 10 min after exercise at 40 and 60 % $\dot{M}_{O_2max}$ (P<0.05). In goats, exercise values were always higher than resting values (P<0.01), and R_a glucose returned to resting levels immediately after completion of the exercise (P>0.05).

Circulatory glucose oxidation

In goats, rates of oxidation of glucose from the circulation also increased with exercise (P<0.01), but reached a plateau at 40 and 60 % $\dot{M}_{O_2max}$. Furthermore, R_a was always higher than R_d during exercise, causing a gradual increase in plasma glucose concentration. The reverse was true during the recovery period, and plasma glucose concentration decreased (Figs 2–4, top and bottom panels). Maximal rates increased from 51 to 85 μmol kg^{-1} min^{-1} as exercise intensity increased (Table 2). In contrast, the maximal rate in dogs was independent of exercise intensity, at approximately 100 μmol kg^{-1} min^{-1} (Fig. 5). The maximal rate of oxidation of circulating glucose was only 1.2 times higher in the dog than

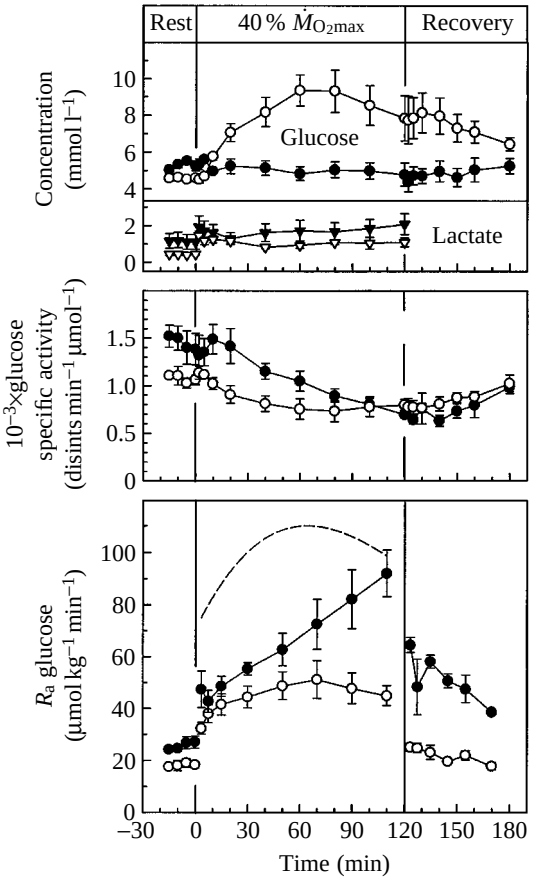

Fig. 2. Plasma concentration, specific activity and rate of appearance (R_a) of glucose before, during and after a 2 h run at 40 % $\dot{M}_{O_2max}$ (circles). Plasma lactate concentration is also given in the top panel (triangles). Dogs (filled symbols, N=3) and goats (open symbols, N=4) received a primed continuous infusion of D-[3-^{3}H]glucose starting 1 h before running, and the infusion rate was increased by a factor 1.8 during exercise. The dashed line in the bottom panel represents 2.2 times R_a glucose of goats ($\dot{M}_{O_2}$ dog/$\dot{M}_{O_2}$ goat=2.2). Values are means ± S.E.M.

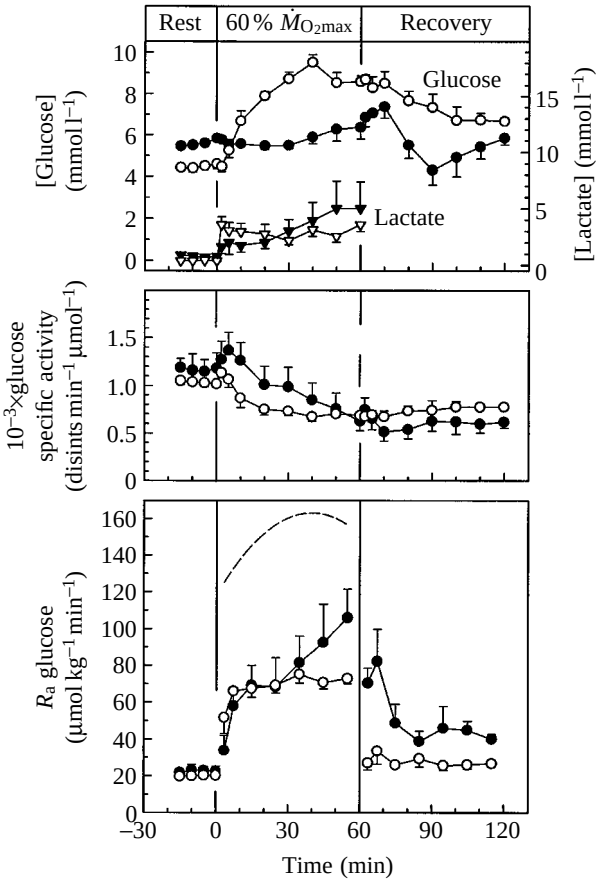

Fig. 3. Plasma concentration, specific activity and rate of appearance (R_a) of glucose before, during and after a 1 h run at 60 % $\dot{M}_{O_2max}$. Infusion rate was increased by a factor 2.4 during exercise. Other details are the same as in Fig. 2. Some error bars have been omitted for clarity.

Table 1. *Plateau lactate concentrations in the plasma of dogs and goats at rest and during exercise at different intensities*

Exercise intensity	Dogs	Goats
Rest	0.8±0.1	0.4±0.1
40 % $\dot{M}_{O_2max}$	1.7±0.1	1.1±0.1
60 % $\dot{M}_{O_2max}$	3.0±0.3	3.0±0.5
85 % $\dot{M}_{O_2max}$	3.4±0.5	11.2±0.2

Means ± S.E.M. (N=3 for dogs, N=4 for goats).

Plateau lactate concentrations (μmol ml^{-1}) are the mean values during the second half of the exercise bout.

in the goat, despite the 2.2-fold difference in rates of total oxidation (Tables 2, 3).

Contribution of circulating glucose to oxidative metabolism

The relative contribution of circulating glucose to total oxidation was small in both species at the exercise intensities we used in these experiments, but it was much higher in goats than in dogs (P<0.0001, Fig. 6). In goats, maximal rates of glucose oxidation could account for only 25, 27 and 18 % of total oxidation at 40, 60 and 85 % $\dot{M}_{O_2max}$, respectively. Corresponding values for dogs were even lower, dropping from 23 % at 40 % $\dot{M}_{O_2max}$ to 16 % at 60 % $\dot{M}_{O_2max}$ and then to 10 % when exercise intensity reached 85 % $\dot{M}_{O_2max}$.

In contrast, maximal rates of oxidation from intramuscular glycogen stores increased dramatically with exercise intensity (P<0.0001). It was this fuel that accounted for most of the increase in oxidation with increasing exercise intensity (Fig. 7), and the oxidation of intramuscular glycogen stores explained most of the difference in maximal rates of oxidation between dogs and goats.

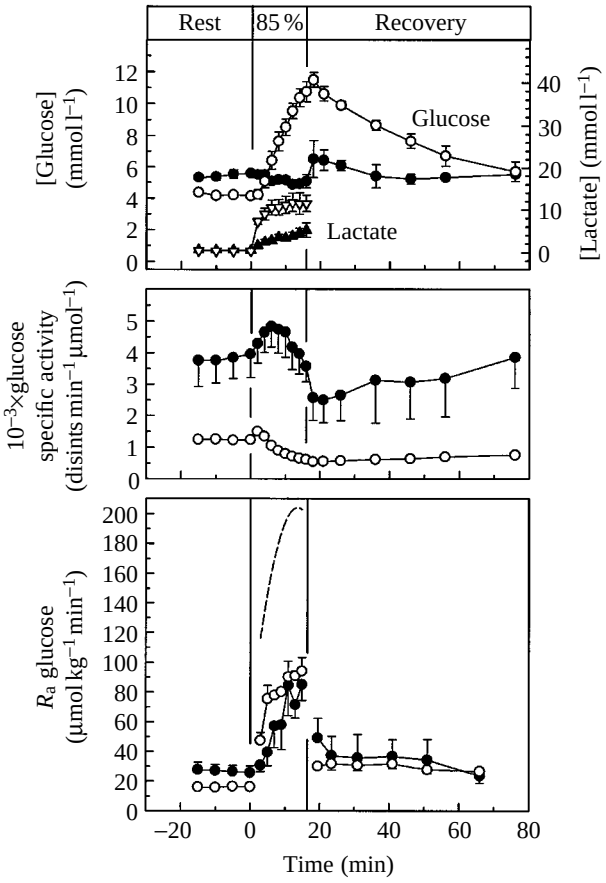

Fig. 4. Plasma concentration, specific activity and rate of appearance (R_a) of glucose before, during and after a 16 min run at 85 % $\dot{M}_{O_2max}$. Infusion rate was increased by a factor 2.4 during exercise. Other details are the same as in Fig. 2. Some error bars have been omitted for clarity.

Discussion

The importance of intracellular glycogen stores

The branch of the carbohydrate pathway from glycogen stores in muscle cells to mitochondria (Fig. 1) is by far the most important source of carbohydrate fuelling oxidation during intense exercise. The more circuitous pathway, providing glucose from the liver *via* the circulation, accounts for less than one-quarter of all the energy used. Furthermore, this fraction decreases as exercise intensity increases, because almost all of the additional fuel oxidized is supplied from intramuscular glycogen stores. Although the relative importance of this circulatory pathway is greater in goats than in dogs, it still contributes only a small fraction of the total

Table 2. *Mean values for aerobic capacity ($\dot{M}_{O_2max}$) and maximal rates of glucose utilization [R_d glucose = $\dot{M}_{CHO(iv)}$] in trained dogs and goats running at three exercise intensities*

	Dogs	Goats	Dog:goat ratio
$\dot{V}_{O_2max}$ (ml O_2 kg^{-1} s^{-1})	2.43±0.05	1.13±0.04	2.2
$\dot{M}_{O_2max}$ (µmol O_2 kg^{-1} min^{-1})	6503±134	3024±107	2.2
R_d glucose = $\dot{M}_{CHO}$(iv) (µmol glucose kg^{-1} min^{-1})			
at 40 % $\dot{M}_{O_2max}$	92.5±9.0	51.3±6.6	1.8
at 60 % $\dot{M}_{O_2max}$	105.1±15.6	75.7±2.6	1.4
at 85 % $\dot{M}_{O_2max}$	98.5±19.4	84.8±6.2	1.2

Values are means ± S.E.M.; N=3 for dogs, N=4 for goats.

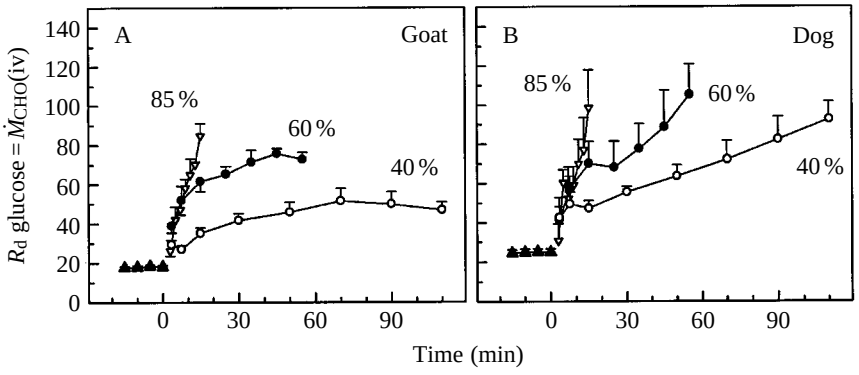

Fig. 5. Rate of glucose disappearance (R_d glucose) at rest and during exercise at 40, 60 and 85 % $\dot{M}_{O_2max}$ in trained goats (A) and dogs (B). Values are means + S.E.M.; N=3 for dogs, N=4 for goats.

Table 3. *Molar oxidation rates per unit body mass in dogs and goats running at three exercise intensities*

Oxidation rate (μmol O_2 kg^{-1} min^{-1})	Dogs			Goats		
	40 % $\dot{M}_{O_2max}$	60 % $\dot{M}_{O_2max}$	85 % $\dot{M}_{O_2max}$	40 % $\dot{M}_{O_2max}$	60 % $\dot{M}_{O_2max}$	85 % $\dot{M}_{O_2max}$
$\dot{M}_{O_2}/M_b$	2392±67	3820±124	5618±146	1184±70	1739±49	2750±162
$\dot{M}_{O_2}^{CHO}$(mt)/M_b	843±128	2221±178	4584±201	296±21	984±67	2222±147
$\dot{M}_{O_2}^{CHO}$(iv)/M_b	555±54	631±94	591±116	308±40	454±16	509±37

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

Values are means ± S.E.M.; N=3 for dogs, N=4 for goats.

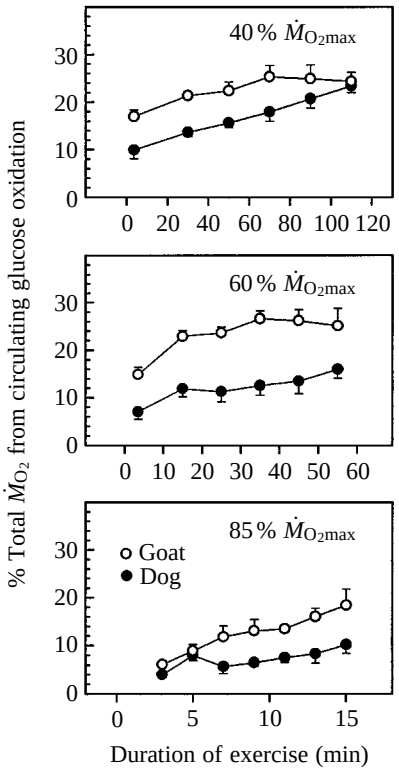

Fig. 6. Percentage contribution of the rate of oxidation of circulating glucose to total energy provision ($\dot{M}_{O_2}$) during exercise at 40, 60 and 85 % $\dot{M}_{O_2max}$ in goats (open symbols, N=4) and dogs (filled symbols, N=3). Values are means ± S.E.M.

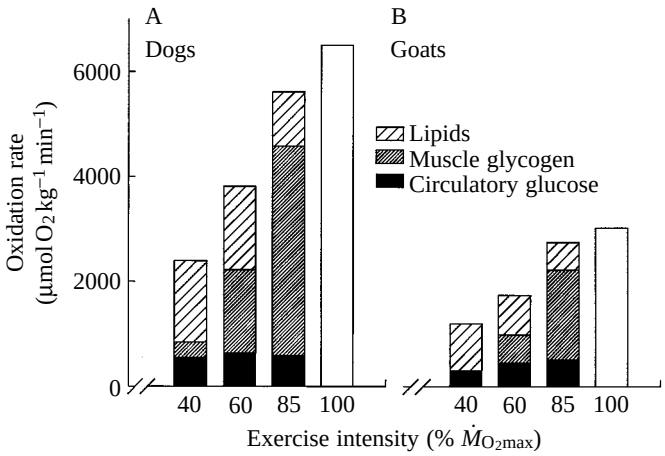

Fig. 7. Rates of oxidation (in μmol O_2 kg^{-1} min^{-1}) of circulating glucose, muscle glycogen and total lipids in dogs (A) and goats (B) exercising at different relative intensities. $\dot{M}_{O_2max}$ is also shown (open bars).

(Fig. 6). Clearly, the pathway from the intramuscular glycogen stores is the primary carbohydrate source in both species when rates of total fuel oxidation are high (Fig. 7).

Circulating glucose as an oxidative fuel

Despite its small relative contribution to total oxidation, the net flux of glucose out of the circulation increases by three- to fourfold during exercise and helps to meet at least some of the increased energy demands of exercise. There is a rapid increase in flux at the onset of exercise in both dogs and goats (Fig. 5). In dogs, the rate of increase then slows, but a plateau is never reached (even during the 2 h of our experiments at low exercise intensity). In this aerobic species, maximal fluxes were reached at the end of the experiments and they were similar at the three exercise intensities (Figs 5, 7). The situation differs in goats. Glucose flux reaches a constant value after about 30 min of exercise. Furthermore, the fluxes are greater at higher exercise intensities (Figs 5, 7), a pattern also observed in humans (Hultman and Harris, 1988).

Glucoregulation

In exercising dogs, the entry of glucose into the circulation is matched to glucose flux out of the circulation, and blood glucose concentration remains constant over the entire range of exercise intensities (Figs 2–4) (Paul and Issekutz, 1967). This is also the case in humans (Coggan *et al.* 1990; Cooper *et al.* 1989; Wasserman and Vranic, 1986; Weber *et al.* 1990). In contrast, blood glucose concentration increases dramatically with exercise intensity in goats, by up to threefold at the 85 % $\dot{M}_{O_2max}$ (Fig. 4). Hyperglycaemia has also been reported in rats, where plasma glucose concentration is proportional to exercise intensity (Sonne and Galbo, 1985). The observation that glucose release into the circulation exceeds the flux out of it in these two sedentary species suggests that it may be a more general phenomenon among sedentary animals.

Liver glycogen stores account for most of the glucose

released into the circulation at the onset of exercise (Wasserman and Cherrington, 1991). However, both glycogenolysis and gluconeogenesis contribute (Weber, 1992) and the importance of gluconeogenesis increases during exercise (Hultman and Harris, 1988). The most important glucose precursor is probably lactate because it is always available from plasma (Figs 2–4; Table 1). Glycerol contributes little to gluconeogenesis, at least in goats where we measured it (Weber *et al.* 1993). Alanine and glutamine are probably the most important amino acids used as glucose precursors during exercise (Wasserman and Cherrington, 1991), but they were not measured in our experiments.

Maximal rates of circulatory glucose oxidation

Our findings suggest that the supply of glucose from the circulation is limited by design constraints in one, several or all of the structural steps in the pathway from blood to mitochondria (Fig. 1). The highest rates of flux do not increase with exercise intensity and they are only slightly higher (about 20 %) in the highly aerobic dog than in the goat. Both of these findings are consistent with a structural limitation. This limitation also appears to exist in other mammals where appropriate measurements have been made (Weber, 1988). Circulating glucose only accounts for a small fraction of total oxidation in rats (Brooks and Donovan, 1983), dogs (Paul and Issekutz, 1967) and humans (Weber *et al.* 1990).

Conclusions

We have drawn the following conclusions.

(1) The supply of energy from muscle glycogen stores increases with exercise intensity, but glucose supply from the circulation does not.

(2) The maximal rate of glucose transport from the circulation to the mitochondria is approximately the same in the dog and the goat (dog:goat ratio 1.2), despite a 2.2-fold difference in their aerobic capacity.

(3) Only a small fraction of the carbohydrate oxidized during exercise is drawn from the circulation (23 % for goats and 13 % for dogs).

(4) Dogs compensate for the shortfall in glucose supply from the circulation by using relatively more glycogen from their intramuscular stores than goats. In addition, the sixth paper of this series (Vock *et al.* 1996) shows that muscle glycogen stores are four times larger in dogs than in goats.

Our findings suggest that the structural design of the branch of the carbohydrate pathway from glycogen stores to mitochondria in the dog has 2–3 times the capacity of that of the goat and that the structural design of the branch from the circulation to the mitochondria is not adjusted to differences in aerobic capacity.

The following paper (Weber *et al*. 1996) addresses the problem of energy provision from circulating and intramuscular fatty acids.

This study was supported by grants from the US National Science Foundation (IBN89-18371), the US National Institutes of Health (AR18140), the Swiss National Science Foundation (31-30946.91) and the Natural Sciences and Engineering Research Council of Canada (OGP0105639).

References

BERGMEYER, H. U. (1974). *Methods of Enzymatic Analysis*. New York: Academic Press.

BROOKS, G. A. AND DONOVAN, C. M. (1983). Effect of endurance training on glucose kinetics during exercise. *Am. J. Physiol.* **244**, E505–E512.

COGGAN, A. R., KOHRT, W. M., SPINA, R. J., BIER, D. M. AND HOLLOSZY, J. O. (1990). Endurance training decreases plasma glucose turnover and oxidation during moderate-intensity exercise in men. *J. appl. Physiol.* **68**, 990–996.

COOPER, D. M., BARSTOW, T. J., BERGNER, A. AND LEE, W.-N. P. (1989). Blood glucose turnover during high- and low-intensity exercise. *Am. J. Physiol.* **257**, E405–E412.

FRIEDMAN, J. E., DOHM, G. L., ELTON, C. W., ROVIRA, A., CHEN, J. J., LEGGETT-FRAZIER, N., ATKINSON, S. M., JR, THOMAS, F. T., LONG, S. D. AND CARO, J. F. (1991). Muscle insulin resistance in uremic humans: glucose transport, glucose transporters and insulin receptors. *Am. J. Physiol.* **261**, E87–E94.

HULTMAN, E. AND HARRIS, R. C. (1988). *Carbohydrate Metabolism.* Basel: Karger. pp. 78–119.

JAHOOR, F., KLEIN, S., MIYOSHI, H. AND WOLFE, R. R. (1988). Effect of isotope infusion and sampling sites on glucose kinetics during euglycemic clamp. *Am. J. Physiol.* **255**, E871–E874.

KATZ, H., HOMAN, M., BUTLER, P. AND RIZZA, R. (1992). Use of [3-^{3}H]glucose and [6-^{14}C]glucose to measure glucose turnover and glucose metabolism in humans. *Am. J. Physiol.* **263**, E17–E22.

KATZ, J. (1992). On the determination of turnover *in vivo* with tracers. *Am. J. Physiol.* **263**, E417–E424.

KATZ, J., ROSTAMI, H. AND DUNN, A. (1974). Evaluation of glucose turnover, body mass and recycling with reversible and irreversible tracers. *Biochem. J.* **142**, 161–170.

LEVY, J. C., BROWN, G., MATTHEWS, D. R. AND TURNER, R. C. (1989). Hepatic glucose output measured with labeled glucose to reduce negative errors. *Am. J. Physiol.* **257**, E531–E540.

MOATES, J. M., LACY, D. B., GOLDSTEIN, R. E., CHERRINGTON, A. D. AND WASSERMAN, D. H. (1988). Metabolic role of the exercise-induced increment in epinephrine in the dog. *Am. J. Physiol.* **255**, E428–E436.

MOLINA, J. M., BARON, A. D., EDELMAN, S. V., BRECHTEL, G., WALLACE, P. AND OLEFSKY, J. M. (1990). Use of variable tracer infusion method to determine glucose turnover in humans. *Am. J. Physiol.* **258**, E16–E23.

PAUL, P. AND ISSEKUTZ, J. B. (1967). Role of extramuscular energy sources in the metabolism of the exercising dog. *J. appl. Physiol.* **22**, 615–622.

RAMAN, M., RADZIUK, J. AND HETENYI, G., JR (1990). Distribution and kinetics of glucose in rats analyzed by noncompartmental and compartmental analysis. *Am. J. Physiol.* **259**, E292–E303.

ROBERTS, T. J., WEBER, J.-M., HOPPELER, H., WEIBEL, E. R. AND TAYLOR, C. R. (1996). Design of the oxygen and substrate pathways. II. Defining the upper limits of carbohydrate and fat oxidation. *J. exp. Biol*. **199**, 1651–1658.

SONNE, B. AND GALBO, H. (1985). Carbohydrate metabolism during and after exercise in rats. Studies with radioglucose. *J. appl. Physiol.* **59**, 1627–1639.

STEELE, R. (1959). Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann. N.Y. Acad. med. Sci.* **82**, 420–430.

TAYLOR, C. R., WEIBEL, E. R., WEBER, J.-M., VOCK, R., HOPPELER, H., ROBERTS, T. J. AND BRICHON, G. (1996). Design of the oxygen and substrate pathways. I. Model and strategy to test symmorphosis in a network structure. *J. exp. Biol.* **199**, 1643–1649.

TURCOTTE, L. P., ROVNER, A. S., ROARK, R. R. AND BROOKS, G. A. (1990). Glucose kinetics in gluconeogenesis-inhibited rats during rest and exercise. *Am. J. Physiol.* **258**, E203–E211.

VISSING, J., SONNE, B. AND GALBO, H. (1988). Role of metabolic feedback regulation in glucose production of running rats. *Am. J. Physiol.* **255**, R400–R406.

VOCK, R. H., HOPPELER, H., CLAASSEN, H., WU, D. X. Y., BILLETER, R., WEBER, J.-M., TAYLOR, C. R. AND WEIBEL, E. R. (1996). Design of the oxygen and substrate pathways. VI. Structural basis of intracellular substrate supply to mitochondria in muscle cells. *J. exp. Biol.* **199**, 1689–1697.

WASSERMAN, D. H. AND CHERRINGTON, A. D. (1991). Hepatic fuel metabolism during muscular work: role and regulation. *Am. J. Physiol.* **260**, E811–E824.

WASSERMAN, D. H. AND VRANIC, M. (1986). *Interaction Between Insulin, Glucagon and Catecholamines in the Regulation of Glucose Production and Uptake During Exercise: Physiology and Diabetes*. Champaign, IL: Human Kinetics Publishers. pp. 167–179.

WEBER, J.-M. (1988). Design of exogenous fuel supply systems: adaptive strategies for endurance locomotion. *Can. J. Zool.* **66**, 1116–1121.

WEBER, J.-M. (1992). Pathways for oxidative fuel provision to working muscles: Ecological consequences of maximal supply limitations. *Experientia* **48**, 557–564.

WEBER, J.-M., BRICHON, G., ZWINGELSTEIN, G., MCCLELLAND, G., SAUCEDO, CHR., WEIBEL, E. R. AND TAYLOR, C. R. (1996). Design of the oxygen and substrate pathways. IV. Partitioning energy provision from fatty acids. *J. exp. Biol.* **199**, 1667–1674.

WEBER, J.-M., KLEIN, S. AND WOLFE, R. R. (1990). Role of the glucose cycle in control of net glucose flux in exercising humans. *J. appl. Physiol.* **68**, 1815–1819.

WEBER, J.-M., ROBERTS, T. J. AND TAYLOR, C. R. (1993). Mismatch between lipid mobilization and oxidation: Glycerol kinetics in running African goats. *Am. J. Physiol.* **264**, R797–R803.

WOLFE, R. R., KLEIN, S., CARRARO, F. AND WEBER, J.-M. (1990). Role of triglyceride–fatty acid cycle in controlling fat metabolism in humans during and after exercise. *Am. J. Physiol.* **258**, E382–E389.

ZAR, J. H. (1984). *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall.