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ENERGY METABOLISM OF THE VIRGINIA OPOSSUM DURING FASTING AND EXERCISE

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Summary

Rates of oxygen consumption and CO_2 production were measured in Virginia opossums (*Didelphis virginiana*) during fasting and prolonged exercise to quantify changes in total energy expenditure and oxidative fuel selection. We hypothesized that fasting would cause metabolic depression and a progressive shift towards lipid utilization to spare alternative substrates. It was also predicted that prolonged exercise would cause the same relative changes in fuel preference as fasting, but on a compressed time scale. The results show that hypometabolism is not used by the Virginia opossum to cope with food deprivation. However, a rapid exhaustion of limited carbohydrate reserves is prevented through a sixfold reduction in the percentage contribution of carbohydrates to total energy expenditure made possible by an increase in lipid utilization. No protein sparing is observed in this species. Prolonged low-intensity exercise elicits a potent mobilization of lipids that allows maximal running time to be extended by delaying the depletion of limited carbohydrate reserves. We conclude that fasting and prolonged low-intensity exercise cause similar changes in the relative use of lipids and carbohydrates, but on a different time scale, supporting the idea that endurance exercise is the metabolic equivalent of 'accelerated fasting'. The absence of metabolic depression and protein sparing during fasting shows that such physiological strategies have not been necessary for the rapid range expansion towards the North recently shown by this species.

Key words: oxidative fuel utilization, lipid, carbohydrate, protein, nitrogen excretion, energetics, food deprivation, indirect calorimetry, energy expenditure, marsupial, Virginia opossum, *Didelphis virginiana*.

Introduction

One of the keys to survival and reproductive success is the ability to adjust rates of energy expenditure and to select adequate metabolic fuels in response to changing environmental conditions. Coping with periods of fasting and exercise can only be achieved if the mobilization of available energy reserves is tightly orchestrated. A reduction in metabolic rate is one of the first lines of defence to prolong survival during food deprivation, a strategy widely used by mammals, even those physiologically incapable of torpor or hibernation (Craven, 1951; Markussen and Oritsland, 1986; Newsholme and Leech, 1983; Nordoy et al., 1990; Taylor, 1975). The ability to endure fasting is enhanced by the progressive mobilization of lipid stores to spare small reserves of carbohydrates and proteins (Cahill, 1970; Le Maho et al., 1987). Interestingly, the gradual changes in relative fuel selection produced by fasting are also usually observed during prolonged locomotion (Terjung and Kaciuba-Uscilko, 1986), although on a much shorter time scale, leading to the idea that endurance exercise is metabolically equivalent to 'accelerated fasting'.

The Virginia opossum (*Didelphis virginiana*) has been one of the most successful mammalian species in recent history, as demonstrated by the dramatic expansion of its range from central America to southern Canada (Austad, 1988). However, it is not known whether fasting-induced hypometabolism has been a necessary physiological mechanism for the ecological success of this species. Marsupials in general (Dawson and Hulbert, 1970; MacMillen and Nelson, 1969; McNab, 1978) and *D. virginiana* in particular (Fournier and Weber, 1994) already have much lower resting rates of energy expenditure than placental mammals, and the opossum may not be capable of further metabolic depression when exposed to food restriction. Moreover, it may not be exposed to long periods of fasting in the wild because of its opportunistic feeding pattern and omnivorous diet (Gardner, 1982). Previous studies have shown that it relies mostly on carbohydrates to support short-term aerobic locomotion (<30 min) even though large lipid reserves are readily available (Fournier and Weber, 1994). Like many other mammals, including humans (McClelland et al., 1999; Shaw et al., 1975; Weber et al., 1993; Wolfe et al., 1990), the opossum may have the capacity for a much stronger stimulation of lipid metabolism when exercising for several hours, but its metabolic response to such a stress has never been measured. Therefore, the goal of this study was to

quantify the effects of fasting and of prolonged submaximal exercise on energy expenditure and oxidative fuel selection in *D. virginiana*, a marsupial species that does not use torpor (Gardner, 1982). We hypothesized that fasting would decrease metabolic rate and cause a progressive shift towards lipid utilization to spare alternative substrates. It was also predicted that endurance exercise would cause the same relative changes in metabolic fuel preference as fasting, but on a compressed time scale.

Materials and methods

Animals

Young wild-caught Virginia opossums *Didelphis virginiana* Kerr were obtained from Arcadia, Florida, USA. They were kept in individual cages (70 cm×45 cm×60 cm), allowed continuous access to water and fed dry puppy chow (Ralston Purina, Canada), apples and bananas. The animals were maintained at 24±1 °C, at 60 % relative humidity, and on a reverse 12 h:12 h light:dark photoperiod with the light on between 22:00 and 10:00 h. Fasting experiments were carried out on six animals (three females and three males), 8 months after their arrival, when they had reached adult size (3.80±0.48 kg, mean ± S.E.M., N=6). Exercise experiments were carried out on juvenile opossums (body mass 1.34±0.02 kg for low-intensity exercise and 1.80±0.03 kg for high-intensity exercise, means ± S.E.M., N=4; two females and two males). These animals were selected from the same litter of nine siblings for their ability to perform prolonged treadmill exercise comfortably. We chose juveniles for the exercise experiments because they can easily run on a treadmill, whereas older opossums do not achieve the same running ability as adults, even when they start their training as juveniles.

Rates of O_2 consumption and CO_2 production

Rates of oxygen consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) were measured using an Oxymax system (Columbus Instruments, Columbus, Ohio, USA) in closed acrylic respirometers (54 cm×38 cm×67 cm) supplied with room air at 3–14 l min^{-1} depending on body mass and experimental protocol (Fournier and Weber, 1994; Weber et al., 1997). A small fan enclosed in the ceiling of the respirometer ensured that the air was well mixed during the measurements. Air flow rate through the respirometer was continuously monitored with a volume-flow regulator accurate to within 1 % of full scale. Oxygen and CO_2 concentrations were measured every 5 min (fasting experiments) or every 30 s (exercise experiments) in the inflow and outflow air, after removing water vapour by passing the air through a calcium sulphate column (Drierite, W. A. Hammond, Xenia, Ohio, USA). The O_2 (electrochemical sensor) and CO_2 analyzers (infrared sensor) were calibrated before measurements (exercise experiments) or once every 24 h (fasting experiments) with known reference gas mixtures. All $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values were corrected for dry gas under standard temperature and pressure (STP) conditions. Gas analyzers and data acquisition were controlled by a personal computer. The measuring system was found to be accurate to within ±2.5 % by bleeding known rates of CO_2 and N_2 or to within ±1.8 % by burning known amounts of ethanol within the respirometer.

Fasting experiments

The animals were transferred to the respirometer 48 h before starting the measurements to familiarize them with the experimental apparatus. After this acclimation period, rates of O_2 consumption and CO_2 production were monitored for 6 days. The animals were fed during acclimation and during the first 3 days of measurements, but they were fasted for the last 3 days. They had continuous access to water throughout the experiments. Measurements were interrupted every day for 30 min at 14:00 h, 2 h into the opossums' active cycle, to calibrate the analyzers and to weigh the animals. The respirometer floor was modified to allow the collection of urine in a container placed on ice. The volume of urine produced was measured every 24 h, and a daily subsample was frozen to measure urinary nitrogen. The total nitrogen content of the urine was quantified in duplicate using the Kjeldahl method (Tecator analyzers, 1007 Digester and Kjeltec System 1002 distilling unit).

Exercise experiments

All exercise measurements were carried out during the dark cycle when this nocturnal species is normally active in the wild. For 1 month before the experiments, the animals were trained 3–5 times a week to run on a horizontal, motorized treadmill enclosed in the respirometer. Measurements were carried out in the post-absorptive state, 18 h after the last meal. Each animal was transferred to the treadmill 1 h before measurements. Pre-exercise resting values were then obtained for 5 min before starting an exercise protocol. Two exercise protocols of different intensity and duration were used (0.2 m s^{-1} for 2 h and 0.5 m s^{-1} for 30 min), and every animal was measured twice for each protocol. Successive measurements using the same animal were always separated by more than 2 days without exercise.

Calculations and statistical analyses

Rates of lipid, carbohydrate and protein oxidation were calculated from $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and nitrogen excretion using the equations of Frayn (1983). For the exercise experiments, nitrogen excretion could not be measured over such a short period, and the mean 24 h value of 0.13 mg N kg^{-1} min^{-1} obtained in post-absorptive animals was used for all individuals in the calculations. In the fasting experiments, statistical differences were assessed using analysis of variance with repeated measures (ANOVA), with time and individual animal as the main factors. Changes in respiratory quotient over time were tested with a non-parametric ANOVA (Kruskal–Wallis) because the variances were not homogeneous. All percentages were transformed to the arcsine of their square root before analysis. Results from the low-

intensity exercise experiments were assessed by linear regressions, but only for values between 30 and 120 min of exercise. All values given are means ± S.E.M.

Results

Fasting experiments

Mean body mass decreased from 3.80±0.48 kg (N=6) at the beginning of the experiment to 3.49±0.45 kg (N=6) after 3 days of fasting (P<0.001). Fig. 1 shows $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and respiratory exchange ratio (RER) for 3 days with access to food and 3 days of fasting. The areas under the $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$ curves (top and middle panels of Fig. 1) were averaged for each day to assess the effect of fasting on overall energy expenditure. Mean daily area under these curves showed no significant decline over time (P>0.05) and, therefore, no metabolic depression was observed (areas under the $\dot{V}_{O_2}$ curve were 9145± 573 ml O_2 kg^{-1} day^{-1} in fed animals and 10 125± 802 ml O_2 kg^{-1} day^{-1} during fasting). Areas during periods of light and darkness were also analyzed separately over time to detect potential changes that had occurred only at night or only during the day, but no significant effect of fasting on energy expenditure was observed (P>0.05). Daily RER showed an overall decline (P<0.001), and mean daily values during feeding (days 1–3) were all significantly higher than daily means during fasting (days 4–6) (P<0.05).

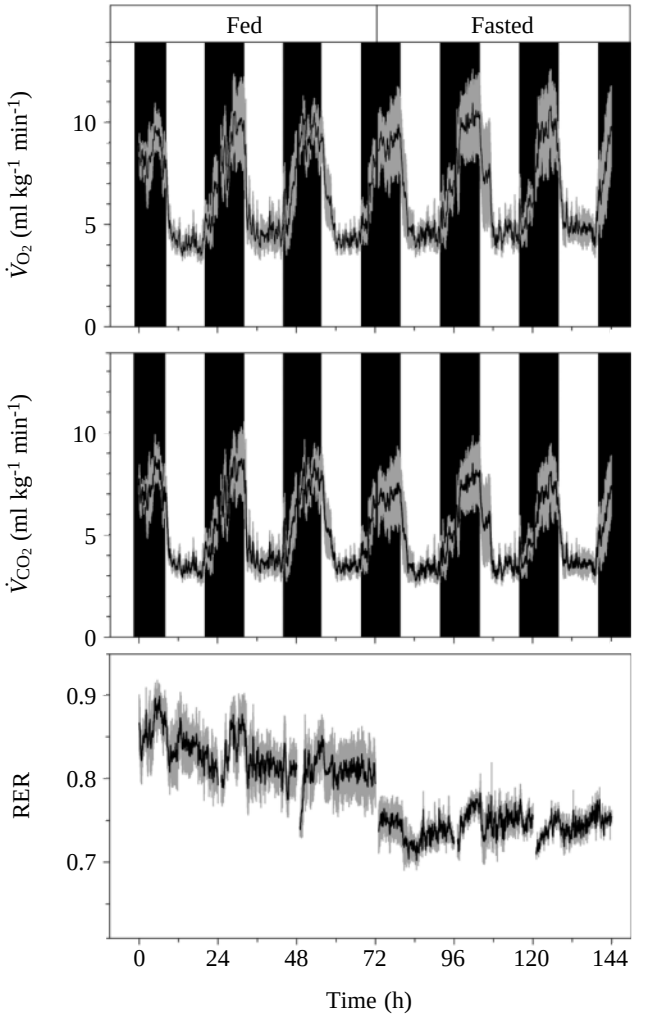

Fig. 1. Daily changes in rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) and in respiratory exchange ratio (RER) of adult Virginia opossums (*Didelphis virginiana*) measured for 6 days (3 days fed and 3 days fasted). Light and dark vertical zones indicate photoperiod. Data were collected every 5 min for 24 h a day throughout the experiments except for a 30 min break each day when the animals were weighed and the analyzers calibrated. Values are means (dark lines) ±S.E.M. (shaded areas) for six animals.

The rate of nitrogen excretion (Fig. 2) showed a significant increase over time (P<0.05) and differed among individuals (P<0.01). Fig. 3 shows absolute rates of lipid (top), carbohydrate (middle) and protein oxidation (bottom). Fasting caused an overall increase in the rates of lipid oxidation (P<0.01) and protein oxidation (P<0.05) and an overall decrease in the rate of carbohydrate oxidation (P<0.001). The oxidation rates of lipids, carbohydrates and proteins were significantly different among individuals (P<0.01). The relative contributions of lipid, carbohydrate and protein oxidation to total oxygen consumption are summarized in Fig. 4. The percentage contribution of lipids increased from 45.1±7.2 to 75.8±2.4 % of $\dot{V}_{O_2}$ (N=6, P<0.001), whereas the contribution of carbohydrates decreased from 43.6±6.0 to 7.4±1.1 % (N=6, P<0.001) throughout the experiments. The percentage contribution of protein oxidation to $\dot{V}_{O_2}$ was 11.3±1.5 % at the beginning and 16.8±2.7 % at the end of the experiments, but these values were not significantly different (N=6, P=0.34).

Exercise experiments

Mean values for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and RER during 2 h of low-intensity exercise at 0.2 m s^{-1} are shown in Fig. 5. Linear regressions on values between 30 and 120 min of exercise reveal that $\dot{V}_{O_2}$ was stable (P>0.05) but that $\dot{V}_{CO_2}$ decreased significantly (P<0.001). Prolonged low-intensity exercise also caused a progressive decline in RER (P<0.001) from a

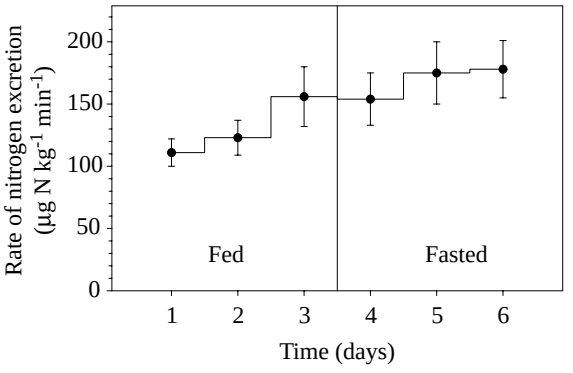

Fig. 2. Rates of nitrogen excretion of adult Virginia opossums (*Didelphis virginiana*) (3 days fed and 3 days fasted). Values are means ± S.E.M. (N=6).

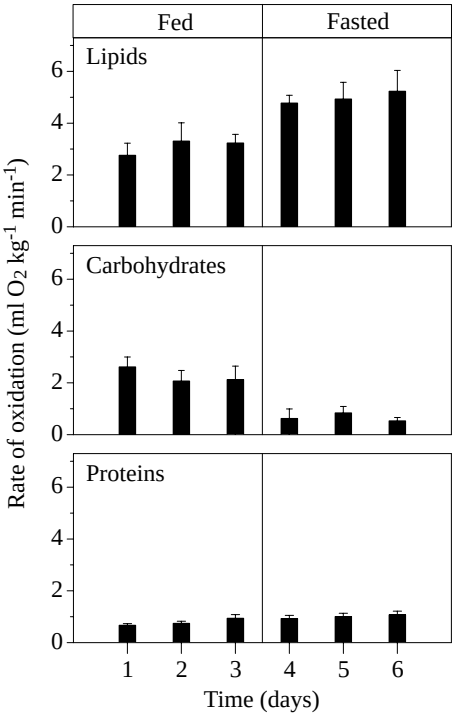

Fig. 3. Absolute rates of lipid (top), carbohydrate (middle) and protein oxidation (bottom) in adult Virginia opossums (*Didelphis virginiana*) (3 days fed and 3 days fasted). Values are means + S.E.M. (*N*=6).

maximal value of 0.957±0.012 after 12 min of exercise to a minimal value of 0.823±0.027 at the end of exercise.

Fig. 6 plots changes in the rates of carbohydrate and lipid oxidation throughout the 2 h of low-intensity exercise. The rate of carbohydrate oxidation decreased from a maximal value of 16.79±1.75 ml O_2 kg^{-1} min^{-1} at 22 min of exercise to 8.16±2.08 ml O_2 kg^{-1} min^{-1} at the end of exercise (P<0.001). The rate of lipid oxidation showed a progressive increase

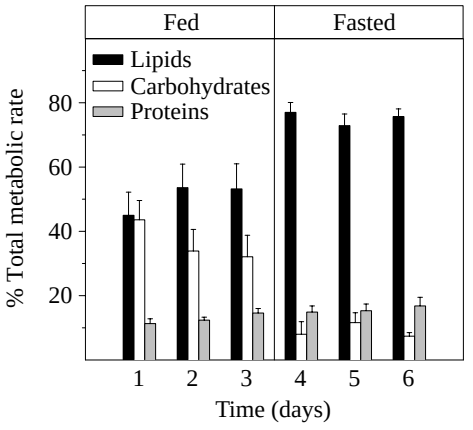

Fig. 4. The energy budget of adult Virginia opossums (*Didelphis virginiana*) (3 days fed and 3 days fasted). Values are mean (+S.E.M.) relative rates of lipid, carbohydrate and protein oxidation given as a percentage of total metabolic rate (*N*=6).

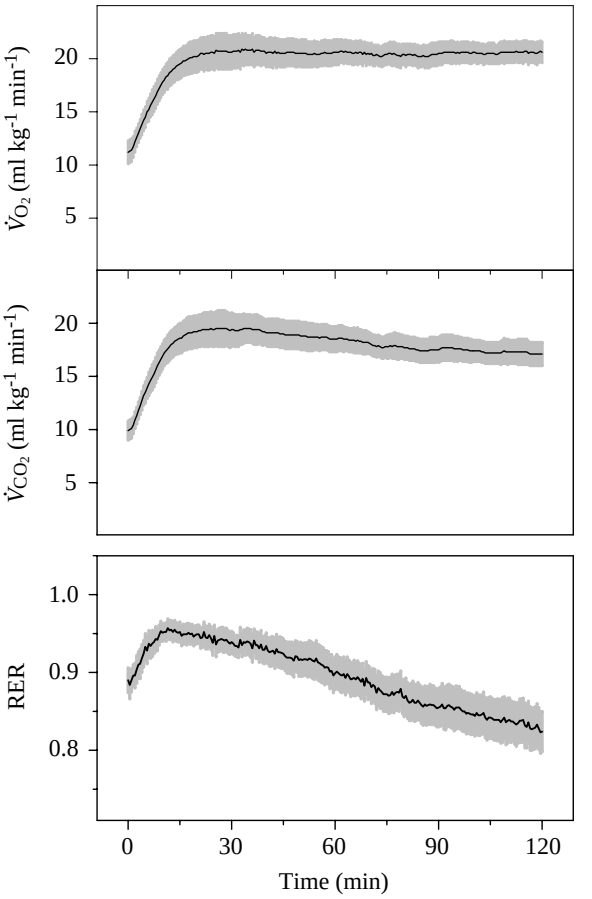

Fig. 5. Changes in rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) and in respiratory exchange ratio (RER) of Virginia opossums (*Didelphis virginiana*) during 2 h of low-intensity exercise at 0.2 m s^{-1}. Values are means (dark lines) ±S.E.M. (shaded areas) (*N*=8 measurements).

of more than fivefold from a minimal value of 2.23±0.77 ml O_2 kg^{-1} min^{-1} measured after 10 min of exercise to a maximum of 11.70±1.88 ml O_2 kg^{-1} min^{-1} at the end of exercise (P<0.001).

Mean values for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and RER during 30 min of high-intensity exercise at 0.5 m s^{-1} are presented in Fig. 7. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased rapidly at the beginning of exercise and stayed at approximately three times resting levels until the end of exercise. RER reached a maximal value of 1.106±0.003 after 8 min of high-intensity exercise before declining progressively to 1.052±0.003 by the end of exercise.

Discussion

Absence of metabolic depression during fasting

Several potential reasons can be proposed to explain why *D. virginiana* does not enter a hypometabolic state during fasting. First, it is possible that post-absorptive marsupials already show the lowest basal metabolic rate attainable by mammals at normal body temperatures. Their known 30 % saving in resting energy expenditure compared with placental mammals

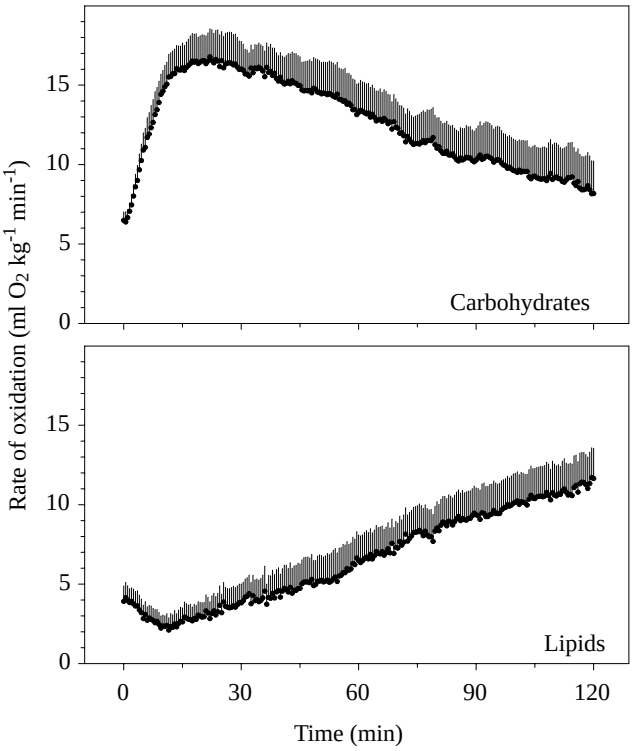

Fig. 6. Changes in rates of carbohydrate and lipid oxidation of Virginia opossums (*Didelphis virginiana*) during 2 h of low-intensity exercise at 0.2 m s^{-1}. Values are means + S.E.M. (*N*=8 measurements).

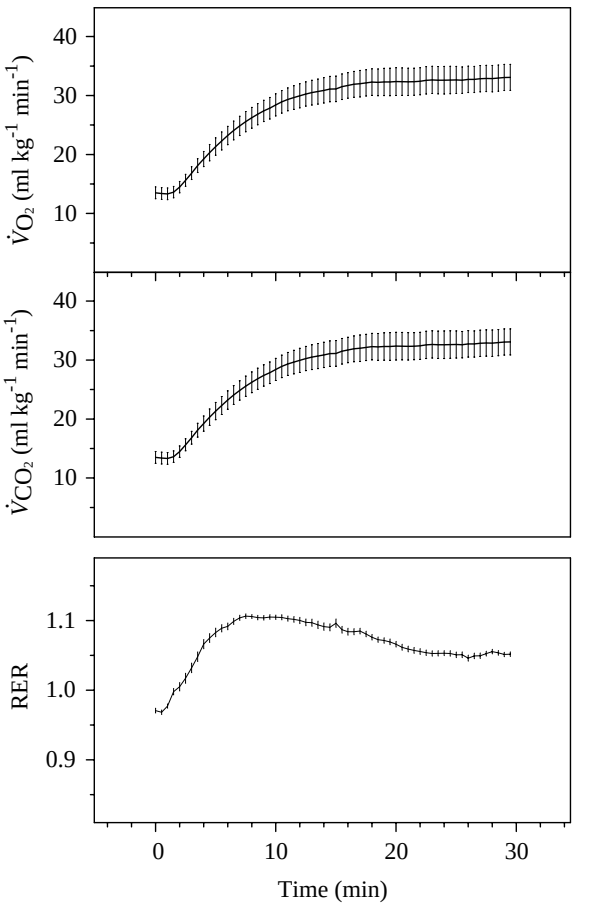

Fig. 7. Changes in rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) and in respiratory exchange ratio (RER) of Virginia opossums (*Didelphis virginiana*) during 30 min of high-intensity exercise at 0.5 m s^{-1}. Values are means ± S.E.M. (*N*=8 measurements).

(Fournier and Weber, 1994) may be achieved by lowering key maintenance functions (e.g. transmembrane ion pumping; Hochachka, 1986) to minimal levels for a normothermic mammal, making any further reduction in metabolic rate impossible without decreasing body temperature and entering torpor (McNab, 1983). If this is true, even desert marsupials may be incapable of normothermic metabolic depression during fasting, and future respirometric studies of desert-adapted marsupials, together with measurements of ion fluxes across marsupial cell membranes, will be needed to resolve this issue.

Another possible way to lower metabolic rate during fasting is to decrease the size of some organ systems (e.g. the digestive and reproductive organs; Blank and Desjardins, 1985; Bronson, 1989), but our results show that the Virginia opossum does not seem to use this mechanism. Taken together, these observations suggest that this species may never be naturally exposed to prolonged periods of food deprivation, possibly because of its ability to survive on a very wide variety of food items (Gardner, 1982). We cannot totally exclude the possibility that *D. virginiana* is capable of fasting-induced metabolic depression but that it takes more than 3 days to initiate such a response. However, this scenario is unlikely because other mammalian species that use hypometabolism show a significant reduction in energy expenditure after a much shorter period without food: less than a day for laboratory rats (Markussen and Oritsland, 1986; Cumming and Morrison, 1960) and seals (*Halichoerus grypus* and *Phoca groenlandica*) (Nordoy et al., 1990; Worthy and Lavigne, 1987) or just a few hours for desert-adapted species such as Bedouin goats (Choshniak et al., 1995) and spiny mice (*Acomys russatus*) (Merkt and Taylor, 1994).

Changes in fuel selection during fasting

Fasting for 3 days caused a sixfold reduction in the relative use of carbohydrates (from 44 to 7 % of $\dot{V}_{O_2}$) that was compensated by an increase in the rate of lipid catabolism (from 45 to 76 % of $\dot{V}_{O_2}$) (Figs 3, 4). This integrated response in fuel selection is absolutely necessary because the Virginia opossum stores enough carbohydrates to survive for only a few hours, whereas its large lipid reserves can sustain life for at least a week (Fournier and Weber, 1994). A shift in lipid and carbohydrate preference of similar magnitude is known to occur in most birds and mammals, but it is usually accompanied by a decrease in protein utilization to protect this critical substrate until shortly before death occurs (Cahill,

1970; Le Maho et al., 1987; Young and Scrimshaw, 1971). Protein sparing is most pronounced in animals that routinely face prolonged periods of food deprivation (penguins, Cherel and Le Maho, 1985; seals, Nordoy et al., 1990; hibernating mammals, Harlow, 1995; Nelson, 1980), but it is also observed in the laboratory rat (Cherel et al., 1992) and in humans (Cahill, 1970). In the present study, the absence of even a blunted tendency to save proteins suggests that the opossum is poorly adapted for prolonged fasting.

Effects of exercise on fuel metabolism

At the onset of low-intensity exercise ($0.2\,m\,s^{-1}$), total energy expenditure doubled (Fig. 5), and this change was entirely supported by a threefold increase in the rate of carbohydrate metabolism because the rate of lipid oxidation decreased to half the resting value (Fig. 6). After 15 min, lipid oxidation was progressively stimulated until the end of exercise, when over 50 % of total ATP was provided through fat catabolism. The relative importance of carbohydrates declined gradually throughout exercise, while lipid oxidation became the dominant pathway for energy metabolism. After 2 h of running, the rate of carbohydrate oxidation had decreased to half the maximal value reached at approximately 20 min. Therefore, this coordinated response in fuel metabolism allows the animal to run for at least twice as long because total carbohydrate reserves are known to limit maximal endurance time in *D. virginiana* (Fournier and Weber, 1994). This finding is ecologically relevant because field measurements of wild Virginia opossums have shown that they travel at average speeds of approximately $0.3\,m\,s^{-1}$ (Ryser, 1992). Earlier experiments had failed to demonstrate that lipid metabolism can play such an important role because measurements were carried out only during short-term exercise lasting for up to 30 min (Fournier and Weber, 1994). Surprisingly, this rather sedentary mammal shows the same ability as more athletic species for an important mobilization of lipid reserves that avoids the rapid depletion of carbohydrate reserves during more prolonged low-intensity exercise (McClelland et al., 1994, 1998, 1999; Roberts et al., 1996; Wolfe et al., 1990).

Intense exercise ($0.5\,m\,s^{-1}$) caused a threefold increase in metabolic rate, and the animals were able to sustain this intensity for at least 30 min (Fig. 7). RER climbed above 1 shortly after the start of high-intensity exercise and was maintained above this value throughout exercise. This indicates that part of the ATP needed by the locomotory muscles was provided by anaerobic metabolism, and that changes in blood and tissue pH were probably taking place. Unfortunately, such changes precluded the use of $\dot{V}_{CO_2}$ to calculate substrate oxidation because, under these circumstances, the CO_2/bicarbonate pool was not in steady state. Therefore, we could not quantify the respective contributions of lipid and carbohydrate oxidation to total energy expenditure during high-intensity exercise.

Concluding remarks

This study shows that metabolic depression is not used by the Virginia opossum to cope with food deprivation and, therefore, that such an energy-saving strategy has not been necessary for the rapid range expansion recently shown by this species (Austad, 1988). During fasting, rapid depletion of limited carbohydrate reserves is prevented through a sixfold reduction in the percentage contribution of carbohydrates to total energy expenditure made possible by an increase in lipid utilization. However, this marsupial does not show protein sparing, even after 3 days without food. The absence of metabolic depression and of protein sparing suggest that the Virginia opossum is poorly adapted for fasting. Prolonged low-intensity exercise elicits a potent mobilization of lipid stores that allows this animal to extend its maximal running time by delaying the depletion of limiting carbohydrate reserves.

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