LOCOMOTORY ENERGETICS AND METABOLIC FUEL RESERVES OF THE VIRGINIA OPOSSUM

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Summary

Marsupials have lower resting metabolic rates than placental mammals, but it is not clear whether particular species can extend this energetic advantage to locomotion. Some active marsupials have a low cost of locomotion, but other more sedentary species, such as the Virginia opossum, appear to run very inefficiently. Steady-state rates of O₂ consumption (V̇O₂) and CO₂ production (V̇CO₂) were measured at rest and during horizontal treadmill exercise in wild-caught, trained opossums. Average daily V̇O₂ in undisturbed animals was 7.73±0.40 ml O₂ kg⁻¹ min⁻¹ (5.67±0.20 ml O₂ kg⁻¹ min⁻¹ during light and 9.84±0.81 ml O₂ kg⁻¹ min⁻¹ during dark hours, mean ± S.E.M., N=6). Net cost of locomotion ranged between 6.16 and 8.99 J kg⁻¹ s⁻¹ as speed increased and was always higher than for an average mammal of equivalent mass. Net cost of transport decreased as speed increased and was 15–80% higher than for an average mammal. During aerobic locomotion, most of the energy was provided by carbohydrate oxidation, which accounted for 60–95% of V̇O₂ as speed increased. Glycogen and triglyceride reserves were quantified in the major storage depots to estimate potential survival time and travelling distance. Enough metabolic fuel was stored to survive for at least 1 week without eating, and 95% of this energy was in adipose tissue triglycerides. However, maximal travelling distance was less than 2 km because opossum locomotion is mainly supported by carbohydrate reserves, which represented only 4% of the available energy. We conclude that aerobic, ground locomotion of Virginia opossums is associated with two major energetic handicaps because their particularly high cost of transport and the nature of the main oxidative fuel they consume are both incompatible with prolonged locomotion.

Introduction

The resting metabolic rate of marsupials is significantly lower than for placental mammals of the same body mass (Dawson and Hulbert, 1970; MacMillen and Nelson, 1969; Nagy, 1987). This lower rate may allow for the allocation of extra energy towards non-maintenance processes, longer survival on equivalent fuel reserves (Dawson and

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Hulbert, 1970) and the ability to survive on diets of lower nutritional value (McNab, 1986). It is unclear whether marsupials can extend this energetic advantage to locomotion. Increased metabolic efficiency has been observed in several species of the Dasyuridae family, which have lower costs of transport than placental mammals (Baudinette et al. 1976). However, many species from other marsupial families are extremely sedentary and exhibit morphological features poorly adapted for cursorial movement. Observation of a running Virginia opossum (Didelphis virginiana Kerr), the only North American marsupial, suggests that its locomotory costs are higher than for an average mammal, a contention supported by morphometric studies where limb orientation and angle of operation do not appear to be optimal for efficient cursorial locomotion (Jenkins, 1971; Jenkins and Weijs, 1979). A possible low cost of transport for this species is further contradicted by telemetric measurements showing that adults have small natural home ranges (Fitch and Hampton, 1970). In addition, males tend to lose weight during the breeding season when their nightly movements increase and the size of their home range doubles (Ryser, 1992). However, there are no direct measurements of the cost of transport of the Virginia opossum.

Typical mammals store over 80% of their energy reserves as triglycerides and comparatively little as glycogen (Hochachka and Somero, 1984; Newsholme, 1988). To what extent opossums use their lipid stores to support basal metabolism or locomotion is unknown. Furthermore, the total amount of energy available in the different body reserves has not been quantified in this species. Therefore, the goals of this study were: (1) to determine the energetic cost of rest and cursorial locomotion in the Virginia opossum, (2) to quantify the respective contributions of lipid and carbohydrate oxidation to total metabolism at rest and during locomotion, and (3) to estimate maximal survival time and running distance of this species based on its metabolic fuel reserves.

**Materials and methods**

**Animals**

Fifteen young wild-caught Virginia opossums, Didelphis virginiana (11 males and 4 females), were obtained from Arcadia, Florida. Results for males and females were pooled because no sex differences were found. The animals were kept in individual cages (68 cm × 45 cm × 60 cm), had continuous access to water and were fed dry puppy chow (Rahlson Purina, Canada), apples and bananas. They were maintained at 24 ± 1 °C, at 45–50% relative humidity and on a reversed 12 h:12 h L:D photoperiod. Exercise measurements were made during their dark cycle (10:00–22:00 h) when this nocturnal species is normally awake and active. Average body mass was 1.58 ± 0.14 kg (range 0.60–3.39 kg).

**O₂ consumption and CO₂ production**

Rates of oxygen consumption (˙\(V_O_2\)) and carbon dioxide production (˙\(V_CO_2\)) were measured with an EcoOxymax system (Columbus Instruments, Columbus, Ohio) in a closed acrylic respirometer (54 cm × 38 cm × 67 cm) supplied with room air (at a rate of
3–141 min\(^{-1}\) depending on body mass and experimental protocol). A small fan, enclosed in the respirometer lid, circulated the air within the experimental chamber to ensure continuous mixing. Air flow rate through the chamber was kept constant with a built-in mass flow regulator during each experiment. This regulator was accurate to within 1\% of full scale, as calibrated with a reference volume meter (Porter Instruments, Pennsylvania). Oxygen and CO\(_2\) concentrations were measured in the inflow and outflow air after removing water vapour through a calcium sulphate column (Drierite, W. A. Hammond, Xenia, Ohio). The Drierite was exposed to air for 5 min before being placed into the column to avoid absorption of CO\(_2\) from the respirometer air samples. The O\(_2\) (electrochemical sensor) and CO\(_2\) (infrared sensor) analyzers were calibrated before and after each experiment with known gas mixtures. Data were collected by an IBM-compatible computer connected to the analyzers. \(\dot{V}_O_2\) and \(\dot{V}_{CO_2}\) were corrected for dry gas under STP conditions. The experimental system was found to be accurate to within \(\pm 2.8\%\) after bleeding known rates of pure CO\(_2\) and N\(_2\), or within 1.8\% by burning known amounts of 99\% ethanol within the respirometer. These values were obtained by averaging steady-state data collected every 30 s over 15 min for each calibration procedure (\(N=30\)).

**Experimental protocols**

All experiments were carried out at 24±1 °C in post-absorptive animals (18 h after their last meal).

*Rest standing quietly*

This protocol was designed to match the resting measurements used to generate previously published allometric equations for resting \(\dot{V}_O_2\) in mammals (for a review, see Heusner, 1991; Kleiber, 1961; Schmidt-Nielsen, 1990), thereby allowing a fair comparison between our data and predicted values for average mammals of equivalent mass. The opossums were placed individually in the respirometer for 1.5 h. \(\dot{V}_O_2\) and \(\dot{V}_{CO_2}\) measured during the last 30 min of the experimental period were averaged to determine steady-state resting values for each animal. In all cases, mean steady-state values had a coefficient of variation \(\{\text{standard deviation/mean}\} \times 100\}\) smaller than 5\%. All measurements were made in the middle of the dark period when the animals were awake, standing quietly, with no access to food or water.

*24 h spontaneous metabolic rate*

Animals were placed individually in the respirometer for 2 h before measuring \(\dot{V}_O_2\) every 5 min for the next 24 h. Measurements were started in the middle of the dark period (12 h, Fig. 1). The experimenter was able to observe the animal discreetly through a 5 cm×5 cm window when necessary. The opossum’s regular light:dark cycle was maintained during the 24 h measurements. Metabolic rate while sleeping was calculated by averaging \(\dot{V}_O_2\) between hours 1 and 2 (light period; see Fig. 1). The respirometer floor was covered with wood chips, the substratum used in their holding cages, and only water was available *ad libitum*.
Exercise protocols

For 3 months, all animals were trained to run on a horizontal treadmill enclosed in the respirometer. Each opossum was trained for 30 min at least five times a week at different speeds. Six individuals out of 15 were selected for exercise experiments because they were able to run particularly comfortably at constant speed, using their natural gait. Each animal stood quietly on the treadmill for 30 min before exercise. \( \dot{V}_O_2 \) and \( \dot{V}_C_O_2 \) were measured every 30 s for 5 min before exercise and for 30 min while moving at a constant speed. The measured rates reached a plateau during the last 15 min of the run, allowing the determination of steady state \( \dot{V}_O_2 \) and \( \dot{V}_C_O_2 \) for each speed. Steady-state rates were defined as the 5 min period when the coefficient of variation was at its lowest, but never exceeding 5 %. All experiments where these criteria were not met were discarded. Experiments on the same animal were always separated by at least 24 h.

Tissue sampling and analyses

Six animals were killed with an intravenous overdose of sodium pentobarbital (480 mg kg\(^{-1}\) body mass) after an 18 h fast. To calculate total carbohydrate (glycogen) and total lipid (triglycerides) reserves, samples were taken from the liver, heart, non-locomotory muscles (trapezius and diaphragm), locomotory muscles (triceps, gracilis and sartorius) and adipose tissue. Approximately 5 g of each tissue was rapidly excised and freeze-clamped with aluminium tongs pre-cooled in liquid nitrogen. All tissues were sampled and frozen in less than 10 min. The samples were stored at \(-80^\circ C\) until assayed for metabolic fuel concentrations. The opossums were then carefully dissected and the masses of various organs and tissues recorded to determine the per cent of body mass for each tissue. The total mass of the locomotory muscles was obtained by adding the masses of all the individual muscles from the four limbs. All visible adipose tissue was dissected as thoroughly as possible from the whole carcass to estimate total adipose tissue mass.

Triglyceride concentration

0.75 g of each frozen tissue sample was crushed in liquid nitrogen with a pre-cooled mortar and pestle. The samples were homogenized (Polytron homogenizer) in a 1:1 chloroform:methanol solution (Folch et al. 1957), and lipids were extracted by vigorous shaking for 20 min at room temperature. After centrifugation, chloroform was added to the supernatant to bring the Folch solution to 2:1. A 0.2 % KCl (w/v) solution was then added to dilute the aqueous phase before discarding it. 3 ml of 99 % ethanol was added to the remaining organic phase before drying it under \( N_2 \). The residue was redissolved in isopropanol, and triglyceride concentration was measured with a diagnostic kit (Sigma, St Louis) by spectrophotometry at 540 nm (Philips PU8600 UV/VIS). Triglyceride standards and blank controls were used with each series of samples to ensure that assay accuracy was maintained.

Glycogen concentration

1 g of each tissue sample was crushed, as described above, before adding 8 % ice-cold perchloric acid. After homogenization, two subsamples of the homogenate were
neutralized with KHCO₃ and gently shaken for 2 h at 40 °C in the presence of amyloglucosidase to hydrolyze glycogen (Bergmeyer, 1974). The reaction was stopped by adding perchloric acid (8%) and the samples were centrifuged before measuring glucose concentration in the supernatant. Glucose concentration was determined enzymatically at 340 nm according to Bergmeyer (1974). Glycogen standards and blank controls were run with each series of assays. We found that the commercial amyloglucosidase from *Rhizopus* mould used for this assay (Sigma, St Louis) contained significant amounts of glucose and/or glycogen. The tissue glycogen concentrations were therefore corrected accordingly. For tissues with low concentrations, failure to make this correction would have overestimated glycogen content by up to 30%.

**Calculations**

For each animal and running speed, net oxygen consumption for locomotion (extra O₂ consumed for moving) was calculated by subtracting resting \( \dot{V}_{O_2} \), measured while standing quietly, from total \( \dot{V}_{O_2} \) during exercise (Alexander, 1968). Net oxygen consumption during locomotion for an average mammal was calculated from established allometric equations by subtracting resting \( \dot{V}_{O_2} \) while standing quietly (Schmidt-Nielsen, 1990):

\[
\dot{V}_{O_2}/M_b = 11.27M_b^{-0.250},
\]

where \( M_b \) is body mass, from total \( \dot{V}_{O_2} \) during locomotion (Taylor *et al.* 1982):

\[
\dot{V}_{O_2}/M_b = 31.80M_b^{-0.319}v_g + 18.18M_b^{-0.311},
\]

where \( \dot{V}_{O_2}/M_b \) is in ml O₂ kg⁻¹ min⁻¹, \( M_b \) is in kg and \( v_g \) (speed) is in m s⁻¹. Equation 2 was established by Taylor *et al.* (1982) from exercise measurements in 62 species of terrestrial mammals, excluding lions and kangaroos, and the original constants of equations 1 and 2 have been recalculated to fit the units used in this study. Mass-specific metabolic rate (\( E_{metab}/M_b \)) was obtained by dividing net \( \dot{V}_{O_2} \) of locomotion by the mass of the animal and converting to energy units (1 ml O₂ = 20.1 J) (Blaxter, 1989). The net cost of transport (energetic cost of moving 1 kg of body mass over 1 m) was calculated by dividing mass-specific metabolic rate by the speed (Alexander, 1968). Rates of lipid and carbohydrate oxidation were calculated from measured \( \dot{V}_{O_2} \) and \( \dot{V}_{CO_2} \) using the equations of Frayn (1983), assuming that the contribution of protein oxidation to total energy metabolism was insignificant (Bülow, 1988; Gessaman and Nagy, 1988; Weber, 1992; Weber *et al.* 1993).

Metabolic energy reserves were calculated separately for the three major storage tissues (liver, locomotory muscles and adipose tissue) by multiplying the respective triglyceride or glycogen concentrations by the appropriate tissue mass. For locomotory muscles, average glycogen and triglyceride concentrations were used because no significant differences were observed between the three muscles sampled (sartorius, gracilis and triceps). Potential survival time on energy reserves from each of the three major storage tissues was calculated by dividing the total energy stored as glycogen or triglycerides by the average daily metabolic rate. Similarly, maximum potential distance covered was calculated by dividing the amount of energy stored by the total cost of locomotion for each
speed (see Figs 6 and 7). In these calculations (see Figs 5–7), we have assumed that each tissue and each metabolite was used exclusively, thereby obtaining a maximum theoretical duration or distance achievable on the energy stored in each tissue separately. This analysis compares how much energy is stored in the different fuel reserves, using ecologically relevant units (i.e. survival time and distance travelled). In nature, however, animals oxidize carbohydrates and lipids simultaneously. Therefore, to obtain a more realistic estimate of energetic limitations, we have used the proportion of carbohydrate and lipid utilized, determined by indirect calorimetry (see Fig. 8), to calculate the maximum distance travelled on a mixture of these fuels (see Fig. 9). Total glycogen reserves accessible for locomotion were calculated by adding liver and locomotory muscle stores. Adipose tissue glycogen was not included because it probably cannot be used for locomotion (Frayn et al. 1989). In these calculations, glycogen reserves always determined the total distance covered because they were limiting at all speeds.

**Statistics**

Mean $\dot{V}_O_2$ from the 24 h measurements were compared using a one-way analysis of variance (ANOVA) and Tukey’s test (see Table 1). Relationships for total oxygen consumption (see Fig. 2), net cost of locomotion ($E_{metab}/M_b$; see Fig. 3) and net cost of transport (see Fig. 4) versus speed were analyzed by linear least-squares regressions. The significance of slopes was assessed using ANOVA. Regression lines for an average mammal were generated by matching each measured opossum value with a predicted value for an average mammal of the same mass. Overall differences between opossums and average mammals for net cost of locomotion and net cost of transport were tested using one-way ANOVA. Analysis of covariance (ANCOVA) was used to compare the regression for observed values (opossums) with the corresponding regression for the predicted values (average mammals of same mass). Values are given as means ± S.E.M.

**Results**

**Rest and spontaneous 24 h $\dot{V}_O_2$**

The resting metabolic rate of opossums while standing quietly (1.5 h rest experiments) was $6.19±0.54 \text{ml O}_2 \text{kg}^{-1} \text{min}^{-1}$ ($N=5$). Spontaneous changes in $\dot{V}_O_2$ over 24 h in undisturbed animals are presented in Fig. 1. Mean values for the complete 24 h period, 12 h darkness, 12 h light, and during sleep are given in Table 1. The mean $\dot{V}_O_2$ values for the 12 h light and sleeping periods were significantly lower than the 24 h value ($P<0.05$), while the mean $\dot{V}_O_2$ during the 12 h dark period was significantly higher ($P<0.05$). The area under the curve during darkness (Fig. 1) was greater than the area under the curve during the light ($P<0.001$, Table 1). Mean $\dot{V}_O_2$ was significantly lower while sleeping (24 h experiments) than when standing quietly (1.5 h rest experiments) ($P<0.05$).

**Locomotion energetics**

The relationship between steady-state $\dot{V}_O_2/M_b$ and speed ($v_g$) for the six trained individuals selected for exercise experiments is presented in Fig. 2: $\dot{V}_O_2/M_b = 20.34v_g + 22.7$, where $\dot{V}_O_2/M_b$ is in $\text{ml O}_2 \text{kg}^{-1} \text{min}^{-1}$ and $v_g$ in m s$^{-1}$. The slope was significantly
different from zero ($P<0.02$). The net cost of locomotion ($E_{\text{metab}}/M_b$ in J kg$^{-1}$ s$^{-1}$) for opossums is shown in Fig. 3 (filled symbols) with predicted values for average mammals of the same body mass (open symbols). Overall, opossums had a much higher net cost of locomotion than that of an average mammal (ANOVA, $P<0.001$). The regressions for measured and predicted values had the same slope (ANCOVA, $P=0.35$), but the opossum regression had a significantly higher intercept ($P<0.001$) than that for an average mammal. Mean intercepts were 5.51 J kg$^{-1}$ s$^{-1}$ (opossums) and 2.29 J kg$^{-1}$ s$^{-1}$ (average mammal). Mean slopes were 7.09 and 8.59, respectively. Fig. 4 shows the net cost of transport (J kg$^{-1}$ m$^{-1}$) as a function of speed. The energetic cost to move 1 kg body mass over 1 m was significantly higher for an opossum than for an average mammal ($P<0.001$). The slopes of the regression lines for measured and predicted values were not significantly different ($P>0.05$). The measured net cost of transport for each animal decreased significantly as speed increased ($P<0.01$).

Table 1. Spontaneous metabolic rates of Virginia opossums (1.61±0.21 kg) over 24 h

<table>
<thead>
<tr>
<th></th>
<th>$\dot{V}<em>{O</em>{2}}$ (ml O$_2$ kg$^{-1}$ min$^{-1}$)</th>
<th>Area under curve (ml O$_2$ kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute range over 24 h</td>
<td>3.55–18.00</td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>7.73±0.40</td>
<td>11 150±573</td>
</tr>
<tr>
<td>12 h dark</td>
<td>9.84±0.81</td>
<td>7 080±582</td>
</tr>
<tr>
<td>12 h light</td>
<td>5.67±0.20</td>
<td>4 070±144</td>
</tr>
<tr>
<td>Sleeping</td>
<td>4.64±0.23</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. ($N=6$).
Sleeping values were measured between hours 1 and 2 on Fig. 1.
Metabolic fuel reserves and oxidation rates

Glycogen and triglyceride concentrations of the different tissues are presented in Table 2. Tissue masses and their percentage contribution to total body mass are given in Table 3. Fig. 5 shows calculated survival time to total depletion of each one of the main energy storage tissues (liver, locomotory muscles and adipose tissue) for glycogen and triglycerides, separately. Maximal running distances on glycogen and triglyceride energy reserves from liver, locomotory muscles and adipose tissue are presented in Figs 6 and 7, assuming that each fuel/tissue combination is used completely and exclusively. Absolute rates of carbohydrate and lipid oxidation measured by indirect calorimetry at rest and during locomotion are given in Fig. 8A. Mean respiratory exchange ratios were...
0.77±0.01 (rest), 0.89±0.04 (0.2 m s\(^{-1}\)), 0.90±0.03 (0.3 m s\(^{-1}\)), 0.94±0.04 (0.4 m s\(^{-1}\)) and 0.99±0.02 (0.5 m s\(^{-1}\)). The rate of carbohydrate oxidation increased significantly from low to high speed (\(P<0.01\)), while there was no change in the rate of lipid oxidation (\(P>0.15\)). The same was true for the relative importance of these two oxidative substrates (Fig. 8B). Using the proportions of carbohydrate and fat oxidation determined from indirect calorimetry, maximal predicted travel distances to total exhaustion of carbohydrate reserves are given in Fig. 9, with the respective contributions of glycogen (to depletion) and triglycerides (only to partial depletion until all carbohydrate stores have been used) as a function of running speed.

Table 2. Glycogen (\(\mu\text{mol glucosyl units g}^{-1}\) wet mass) and triglyceride concentration (\(\mu\text{mol g}^{-1}\) wet mass) in tissues of the Virginia opossum

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glycogen</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>247.8±48.5</td>
<td>17.9±2.8</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>71.4±7.3</td>
<td>494.8±31.4</td>
</tr>
<tr>
<td>Locomotory muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sartorius</td>
<td>122.3±13.3</td>
<td>13.6±2.3</td>
</tr>
<tr>
<td>Gracilis</td>
<td>117.4±14.9</td>
<td>11.0±2.9</td>
</tr>
<tr>
<td>Triceps</td>
<td>126.5±7.2</td>
<td>15.4±1.4</td>
</tr>
<tr>
<td>Mean</td>
<td>122.1±6.7</td>
<td>13.3±1.3</td>
</tr>
<tr>
<td>Other tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trapezius</td>
<td>113.3±11.1</td>
<td>8.1±1.8</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>113.0±16.6</td>
<td>18.7±3.2</td>
</tr>
<tr>
<td>Heart</td>
<td>78.0±10.5</td>
<td>2.0±0.4</td>
</tr>
</tbody>
</table>

Values are means ± s.E.M. (\(N=6\)).
Discussion

This study shows that the Virginia opossum is not adapted for prolonged exercise because its net cost of locomotion is 15–80% higher than for an average mammal of the same body mass, depending on speed (Fig. 3). In addition, most of the energy used to power aerobic locomotion comes from carbohydrates (Figs 8 and 9), an oxidative fuel stored in very small quantities relative to lipids (Figs 5–7). The opossum’s high cost of locomotion and strong reliance on carbohydrates are two major handicaps limiting its ability to sustain exercise. Inefficient locomotion may be a correlate of this animal’s body morphology. Its musculature and skeletal elements have been described as non-cursorial (Jenkins, 1971; Jenkins and Weijs, 1979) and probably evolved to favour movement in an arboreal habitat (McNab, 1978). The allometric equation derived from 62 species (Taylor et al. 1982) predicts the energetic cost of an average mammal, anatomically designed for average locomotory efficiency. Morphological differences between species may cause the locomotory costs of certain mammals to deviate substantially from average costs (Fancy and White, 1987). The allometric predictions made here were based solely on mass and did not take body shape or mechanical design into account. Therefore, the high cost of transport observed for the opossum may be a direct consequence of an arboreal

![Bar chart](image)

Fig. 5. Calculated survival time of opossums until complete depletion of glycogen or triglycerides from the main energy storage tissues, assuming that each metabolic fuel/storage area is used exclusively and that the metabolic rate is the 24 h mean value. Values are means ± s.e.m. (N=6).

Table 3. Absolute mass and percentage of body mass of Virginia opossum tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mass (g)</th>
<th>Percentage of body mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>105.0±7.9</td>
<td>2.52±0.20</td>
</tr>
<tr>
<td>Locomotory muscles</td>
<td>354.0±39.1</td>
<td>8.14±0.83</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>478.7±45.2</td>
<td>12.76±1.11</td>
</tr>
<tr>
<td>Whole body</td>
<td>4431±448</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. (N=6).
design that is poorly adapted for level running. This does not mean that cursorial, ground locomotion is unnatural for this species because behavioural studies clearly show that this type of movement is commonly used while searching for food in the wild (Fitch and Hampton, 1970).

Are our results representative of opossums moving normally in nature? To ensure the reliability of metabolic rate measurements during locomotion, we accustomed 15 animals to the experimental apparatus over several months, selected the six individuals showing the best response to this training period and defined stringent criteria for determining

Fig. 6. Maximum calculated travel distances on glycogen reserves of major energy storage tissues as a function of running speed. Note that values for adipose tissue are given here for comparison only, because glycogen from this tissue probably cannot be used for locomotion. Values are means ± s.e.m. (N=6).

Fig. 7. Hypothetical maximum calculated travel distances on triglyceride reserves of major energy storage tissues if Virginia opossums were able to power locomotion exclusively with this oxidative fuel. Values are means ± s.e.m. (N=6).
steady-state \( \dot{V}_O_2 \). Taken together, these precautions minimized the possibility that the animals chosen for exercise measurements were stressed by the experimental protocols. To ensure that the net cost calculations were adequate, particular care was taken to provide a fair comparison of resting \( \dot{V}_O_2 \) between opossums and average mammals. This study and several others (Dailey and Hobbs, 1989; Dawson and Hulbert, 1970; Hayes et al. 1992) show that protocol standardization is crucial for proper comparisons of resting data. Therefore, the same resting protocol was used in our experiments as in studies where allometric equations for average mammals were developed (Dawson and Hulbert, 1970; Kleiber, 1932; Taylor et al. 1982). These standardized resting rates were subtracted from the total \( \dot{V}_O_2 \) to calculate comparable net costs of locomotion and transport. The mass-specific metabolic rates of two exercising individuals of the same species, but of larger body mass, have been reported previously (Crompton et al. 1978). The \( \dot{V}_O_2/M_b \) value of these two opossums was 33% lower than that of our six animals, and the
difference in body mass between the two studies can account for one-third of the difference in $\dot{V}O_2$. The rest of the difference remains unexplained.

Our results show that the net cost of transport decreases as speed increases, making it beneficial for opossums to operate at high speed (Fig. 4). It has been suggested that marsupial locomotory muscles may be less efficient at low than at high contracting velocity (Baudinette et al. 1976). However, no clear biochemical or physiological explanation for such an effect is yet available for this group of mammals. Unfortunately, the option of moving quickly to lower the cost of transport is not available to this species because exercise cannot be sustained at high speed. More specifically, an almost exclusive reliance on small carbohydrate reserves to support rapid locomotion strongly limits the duration of intense exercise (over 90% of the energy comes from carbohydrates at 0.5 m s$^{-1}$; see Fig. 8). Observations of opossums in the wild (Ryser, 1992) and in captivity (McManus, 1970) reveal that their average speed is about 0.3 m s$^{-1}$. Even at this lower speed, over 60% of the ATP used for muscle contraction is derived from the oxidation of carbohydrates (Fig. 8), a metabolic fuel representing less than 4% of total energy reserves (Figs 5–7). Other mammals, including humans, also store the great majority of their energy as lipids (Bülow, 1988; Felig and Wahren, 1975; Terjung and Kaciuba-Uscilko, 1986), but indirect calorimetry measurements clearly show that, unlike opossums, they rely mostly on these fat reserves to power low-speed locomotion (Brooks and Donovan, 1983; Krogh and Lindhard, 1920; McClelland et al. 1994; Saltin and Karlsson, 1971; Wolfe et al. 1990). Opossums would be able to cover 35–80 km (depending on speed) on adipose tissue triglycerides, if they were able to use this energy source exclusively (Fig. 7). Realistic calculations, taking into account the actual fuel mixture used in exercise, reveal that, without feeding, opossums can only travel 1.4–1.8 km before exhausting their carbohydrate reserves (Fig. 9). Interestingly, average overnight travelling distances for non-breeding wild individuals are under 1 km (0.95 km)}
for males and 0.41 km for females) (Gillette, 1980), but males are known to double their range during the breeding season (Ryser, 1992), when they would clearly be operating very close to the limits of their energy reserves.

The resting metabolic rate of Virginia opossums is 30% lower than for placental mammals of the same mass, and this observation is in accordance with published values for the same species (McNab, 1978) and for marsupials in general (Dawson and Hulbert, 1970; MacMillen and Nelson, 1969). The opossum’s low basal metabolic rate and particularly high cost of transport provide compounding energetic reasons for this species to be as sedentary as possible. Our 24h metabolic rate measurements (Fig. 1; Table 1) show that $\dot{V}_O_2$ changes by 2.5-fold between sleep during the light period and their highest ‘activity’ level at the end of the dark period. Metabolic rate increased progressively during the dark period, with more pronounced fluctuations in the second half of that period. On non-experimental days, the animals were normally fed at hour 12 (Fig. 1), and the observed fluctuations may be associated with the search for food. As expected for a nocturnal species, mean dark $\dot{V}_O_2$ was significantly higher than mean light $\dot{V}_O_2$ (Table 1).

The opossum stores enough energy to survive for at least 6 days on adipose tissue triglycerides alone and for an extra 0.5 day on other metabolic reserves combined (Fig. 5). These values are minimal estimates for several reasons. First, the daily metabolic rates measured here were for animals deprived of food for a maximum of 1.5 days. In response to a longer fast, they may be able to decrease their metabolic rate to save energy, as reported in other species (Young and Scrimshaw, 1971), thereby increasing survival time. Second, the animals were briefly fasted before tissue sampling, which means that the energy equivalent of 0.5 day had already been consumed. Third, only the fuels stored in liver, locomotory muscles and adipose tissue were taken into account; the potential contribution of other tissues (e.g. non-locomotory muscles) was not added.

In summary, this study shows that aerobic, ground locomotion of the Virginia opossum is associated with two important metabolic handicaps. Their particularly high cost of transport and the nature of the main oxidative substrate they consume are both incompatible with prolonged movement. These animals store enough energy to survive for at least 1 week without eating, but their maximal travelling distance is less than 2 km because locomotion is powered mainly by the oxidation of very limited carbohydrate reserves.

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References


