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Roles of lactate and catecholamines in the energetics of brief locomotion in an ectothermic vertebrate

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Abstract We have investigated the magnitude and duration of excess post-exercise oxygen consumption (EPOC) in a lizard following a single bout of vigorous exercise of 5–60 s, common activity durations for many ectothermic vertebrates. Desert iguanas (*Dipsosaurus dorsalis*) were run for 5 s, 15 s, 30 s, or 60 s. Oxygen consumption ($\dot{V}O_2$) increased from 0.16 ml O_2 g^{-1} h^{-1} at rest to 1.3–1.6 ml O_2 g^{-1} h^{-1} during 5–60 s of running. EPOC duration increased with activity duration, ranging from 35–63 min. EPOC volume, the excess oxygen consumed post-exercise, doubled from 0.13 ml O_2 g^{-1} following 5 s of activity to 0.25 ml O_2 g^{-1} after 60 s. EPOC represented 91–98% of the total metabolic expense of the activity. EPOC durations were always shorter than the period required for lactate removal, illustrating that these two processes are not causally related. Alpha- and beta-adrenergic receptor blockade by phentolamine and propranolol had no effect on resting $\dot{V}O_2$ but depressed excess post-exercise oxygen consumption volumes 25–40%. The extent of catechol stimulation post-exercise may be motivation or stimulus dependent. The data indicate that metabolic elevations post-exercise represent the majority of activity costs in lizards. The study suggests that EPOC of ectothermic vertebrates is sensitive to exercise duration and catecholamine release post-activity, even when activity periods are less than 60 s in duration.

Key words Exercise · Lactate · Oxygen consumption · Lizard, *Dipsosaurus dorsalis* · Oxygen debt

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Abbreviations *EPOC* excess post-exercise oxygen consumption

Introduction

Brief periods of vigorous activity can have profound effects on an animal's metabolic rate that extend well beyond the period of activity itself. It is well documented in many terrestrial vertebrates that a 10- to 20-fold increase in metabolic rate can accompany the onset of activity from the rested state (Taylor 1977; Taylor et al. 1981, 1982; Bennett 1982). Often overlooked in discussion of activity energetics, however, is that elevated rates of metabolism may persist for significant periods following the cessation of activity. In fact, when animal activity is short in duration, the "excess" oxygen consumed post-activity can far exceed the volume consumed during the activity period itself. Although excess post-exercise oxygen consumption (EPOC) has been documented in both vertebrates and invertebrates (Gaesser and Brooks 1984; Full and Herreid 1984; Powers et al. 1987; Gore and Withers 1990a, 1990b; Gleeson 1991; Harrison et al. 1991; Bahr 1992; Børsheim et al. 1994; Baker and Gleeson 1998, 1999), the magnitude and determinants of this post-exercise elevation have been systematically investigated in few animals other than humans.

Post-exercise oxygen consumption was first documented in humans by Benedict and Carpenter (1910). Subsequently, Hill and Lupton (1923) articulated their "O_2 debt" hypothesis, which stated that an "oxygen deficit" created during the transition from rest to exercise was repaid during recovery. The energetic cost associated with the removal of lactate formed during the O_2 deficit was originally thought to be a major component of the O_2 debt (Hill and Lupton 1923; Margaria et al. 1933). These terms and ideas were readily adopted and have persisted for several decades, although evidence that the oxygen-debt hypothesis was flawed has been available since 1927 (Abramson et al. 1927; see

Gaesser and Brooks 1984 for an historical review of O_2 debt). Several contemporary studies have challenged the appropriateness of perpetuating the terms debt and deficit (Gaesser and Brooks 1984; Cerretelli 1990; Bahr 1992; Gatten et al. 1992). Studies of ectothermic vertebrates have shown that lactate removal and oxygen consumption are not always stoichiometrically or temporally related (Bennett and Licht 1973; Gleeson and Dalessio 1989; Wagner and Gleeson 1996; Gleeson 1991, 1996). Gaesser and Brooks (1984) offered the term EPOC to "avoid the implication of causality in describing the elevation of metabolic rate above resting levels following exercise."

Several physiological factors appear to contribute to EPOC in mammals, including restoration of phosphagen levels (Bahr 1992; Gatten et al. 1992), increased ventilation and heart rate (Bahr 1992), removal of lactate and glycogen replenishment (Brooks and Gaesser 1980; Åstrand et al. 1986), restoration of oxygen levels in hemoglobin and myoglobin (Bangsbo et al. 1990; Gatten et al. 1992), increased body temperature in endotherms (Brooks et al. 1971; Hagberg et al. 1980; Gore and Withers 1990a), and the effects of increased catecholamine levels (Barnard and Foss 1969; Cain 1971; Coulsen and Hernandez 1983; Børsheim et al. 1994). In addition to these physiological factors, exercise duration and intensity also appear to contribute to the magnitude and persistence of EPOC in some mammals (Hagberg et al. 1980; Bahr 1992; Gore and Withers 1990a, 1990b; Zanconato et al. 1991).

Zanconato et al. (1991) studying humans and Baker and Gleeson (1998, 1999) using mice have shown that when activity periods are as brief as 5–60 s, EPOC represents 90–99% of the elevated oxygen consumption associated with activity. This is true in lizards also (T.V. Hancock et al., unpublished observations), an animal for whom brief activity is typical. The potential magnitude of the energetic expense associated with recovery from brief vigorous activity in most ectotherms has prompted us to investigate the EPOC phenomenon in a lizard in which aspects of its recovery metabolism and endocrinology are already known (Gleeson et al. 1993; Wagner and Gleeson 1996; Scholnick et al. 1997). In this report we relate EPOC resulting from brief (5–60 s) exercise to lactate accumulation and its removal and investigate the role of elevated catecholamines on the energetics of recovery from brief exercise. We use these data to test the hypothesis that post-exercise oxygen consumption in lizards is proportional to activity duration.

Materials and methods

Animal care

Desert iguanas, *Dipsosaurus dorsalis*, collected near Palm Springs, Calif. (July–August 1995) with permission from the California Department of Fish and Game, were transported to Colorado. Eleven, non-reproductive females were selected (mass 26.0–36.8 g, mean 30.7 ± 2.52 g) and placed in cages with a 24-h photothermal gradient. Animals were fed twice weekly on a diet of chopped lettuce and powdered rat chow. Animals were fasted 5–7 days before experimentation. Water was provided ad libitum.

Rest and exercise protocol

Exercise was conducted on a motorized, thermostatted treadmill with variable speed control (0–1.1 m s^{-1}). One and a half hours before each exercise bout, animals were fitted with a clear acetate respiratory mask that surrounded the head of the animal and from which expired gases were drawn. The animal was then placed on the treadmill surface and covered with an opaque plastic container to minimize visual disturbance to the animal. Animals were induced to sprint on the treadmill by gently prodding the hindlimbs and tail. Treadmill speed was adjusted by the experimenter to match the speed of the lizard and to maximize locomotor performance. Each animal sprinted for 5 s, 15 s, 30 s, and 60 s. The sequence of sprint duration was determined randomly for each animal. Upon termination of each sprint, each animal was allowed to recover quietly on the treadmill under the opaque box for 3 h. Expired gases were monitored before, during, and after the activity period. Data from animals active during recovery were rejected. No animal was exercised more than once every 2 days, and most animals were fed and refasted between measurements.

For measurement of resting metabolic rate, fasted animals were fitted with masks and kept in a temperature-controlled box for 4 h on 3 non-consecutive days. Expired gases were collected and rates of oxygen consumption ($\dot{V}O_2$) calculated using the methods described below. The lowest 15 min average from each of the three resting periods were averaged to obtain a resting rate of oxygen consumption for each animal.

Measurement of oxygen consumption

Oxygen consumption was measured using a high-flow, minimum-volume system previously described (Baker and Gleeson 1998, 1999). Air flow was regulated by a Tylan RO-32 mass flow controller ($rates = 180–650\, ml STPD\, min^{-1}$, depending upon whether resting or activity $\dot{V}O_2$ was being measured) and analyzers were calibrated with reference gas mixtures before the beginning and at 15-min intervals during each measurement period. Data acquisition was accomplished by a Lab-NB data acquisition board (National Instruments, Austin, Tex.) using LabVIEW (v3.1.1, National Instruments) programs written to compute, display, and record instantaneous $\dot{V}O_2$ using Eq. 4b of Withers (1977) following "instantaneous" corrections of expired O_2 and CO_2 according to Eq. 3 of Bartholomew et al. (1981). Data acquired at approximately 100 Hz were averaged over 1-s intervals during measurement of activity $\dot{V}O_2$ and during the first 15 min of recovery periods, and averaged over 5 s during measurement of resting $\dot{V}O_2$ and late recovery $\dot{V}O_2$. The response time of the system at a flow rate of 650 ml min^{-1} to a full scale step change in gas concentration at the mask was 1–1.5 s.

Calculation of EPOC volume and duration

EPOC was determined by comparing the post-exercise metabolic rate of the lizard to the resting rate of that animal in a manner consistent with previous studies (Baker and Gleeson 1998, 1999). The end point of recovery was defined as occurring when post-exercise $\dot{V}O_2$ averaged over 5 min equaled or fell below two standard deviations above the sample mean standard metabolic rate. In other words, we interpreted the endpoint of recovery as occurring when recovery $\dot{V}O_2$ fell within the approximate 95% confidence interval of $\dot{V}O_{2,\,rest}$ for these animals. For animals used to measure the rate of lactate removal, the endpoint of recovery reported was determined by fitting the recovery $\dot{V}O_2$ to a double exponential through a reiterative fit process (Kaleidagraph, Abelbeck Software)

and then using the predictive equation to estimate the 95% recovery time. To determine the EPOC volume consumed after each sprint, the average $\dot{V}O_2$ over the recovery time period was multiplied by the recovery time as follows:

$$EPOC\ volume = (\dot{V}O_{2,\,EPOC} - \dot{V}O_{2,\,rest}) \cdot t_{EPOC} \quad (1)$$

where EPOC volume has the units of ml O_2 g^{-1}, $\dot{V}O_{2,\,rest}$ have units of ml O_2 g^{-1} h^{-1}, and t_{EPOC} is in hours. The volume of excess oxygen consumed during exercise, or the EEOC volume, was calculated in an analogous manner:

$$EEOC\ volume = (\dot{V}O_{2,\,exer} - \dot{V}O_{2,\,rest}) \cdot t_{exer} \quad (2)$$

where EEOC is excess exercise oxygen consumption in ml O_2 g^{-1}, and $\dot{V}O_{2,\,exer}$ is the integrated average rate of oxygen consumption during the exercise period.

Blood lactate

Each animal ($n=6$) was fitted with an occlusive carotid cannula under halothane anesthesia and allowed to recover for 48 h. Animals were placed individually in a temperature controlled cabinet (40 ± 1.0°C) 4 h before the sprint occurred. The animals were quickly transferred to the treadmill and induced to sprint for 5 s, 15 s, or 60 s. Exercise duration was selected in random order for each animal with at least 24 h between sprints. The animals were then removed from the treadmill and placed back into the container in the temperature controlled cabinet. A cannula extension of PE50 tubing was added and threaded through an opening in the container to allow sampling without visually disturbing the animal. Blood samples of 25 µl were taken after exercise and at intervals of 5 min, 30 min, and 60 min post-exercise. Blood samples were added to four volumes of 6% perchloric acid ($HClO_4$) and stored at –70°C. Lactate concentrations were determined in supernatants according to Gleeson (1985) and are reported as millimoles lactate/liter whole blood. Respiratory gas exchange during this experiment was monitored to allow comparison of recovery under these experimental conditions to that of uncannulated animals.

To obtain resting blood samples, the animals were placed in the 40°C cabinet and allowed to rest for 4 h. At the end of this time period, a blood sample (25 µl) was quickly taken via the orbital sinus with a capillary tube. Samples that took longer than 30 s to acquire or samples where the animal struggled were discarded. Preliminary experiments confirmed that resting samples taken via orbital sinus were no different than those drawn from cannulae. Blood samples were treated as described above.

α- and β-adrenergic blockade

To determine the role that the elevated catecholamines after activity (Gleeson and Dalessio 1989) may have on EPOC, blockers of α- and β-adrenergic receptors were administered in separate experiments. In these experiments, each lizard ($n=8$) was placed in a black polyvinyl chloride (PVC) tube placed on the surface of the temperature regulated (40 ± 1.0°C) treadmill. Air was drawn across the animal through the tube and then sampled as described above. The animal was allowed to adjust to the tube for 2.5 h. Five min prior to activity, the animal was quietly removed from the tube and injected i.p. with a 5 µl/g volume of either 48.3 or 483 nmol g^{-1} body weight propranolol (a β-blocker, Sigma P0884) in saline, 34.9 or 349 nmol g^{-1} body weight phentolamine (an α-blocker, Sigma P7547) in saline (5 µl/g bw), a combination of both blockers, or with saline solution alone. After injection, the animal was placed back in the tube. Five min post-injection, the animal was taken from the tube and induced to sprint for 5 s, 15 s, 30 s, or 60 s on the treadmill. Immediately after the sprint, the animal was placed back into the tube and allowed to recover quietly for the remainder of 80 min. To determine the effects of adrenergic blockade on resting metabolic rate, drugs or saline were administered without subjecting the animal to exercise.

Statistical analysis

Repeated measures analysis of variance (ANOVA) and Student's paired *t*-tests were used to distinguish differences between the measured variables with sprint duration as the independent value. For repeated paired *t*-tests, critical α value was adjusted downward by the Dunn-Sidak method (Sokal and Rohlf 1994) so as to keep experiment-wise error at 5% according to the equation $0.05 = 1 - (1 - \alpha)^{1/k}$, where α equals the critical *p* value and *k* equals the number of paired comparisons. Statistical analysis was performed using Statview v4.5 (Abacus). Data are reported as mean ± 1 SEM.

Results

Exercise and recovery oxygen consumption

Resting animals exhibited a mean rate of oxygen consumption of 0.157 ± 0.016 ml O_2 g^{-1} h^{-1} ($n=11$). V_{O2} rapidly increased during treadmill sprinting, and reached peak maximal values within 5 s (Fig. 1). The metabolic rates of sprinting animals ($n=11$) were elevated 8- to 10-fold over that of resting animals. Oxygen consumption averaged over the entire exercise period ($\dot{V}O_{2,\,exer}$) increased slightly with sprint duration, from 1.31 ml O_2 g^{-1} h^{-1} during 5 s of running to 1.49 ml O_2 g^{-1} h^{-1} during 60 s of running. However, there were no statistical differences in $\dot{V}O_{2,\,exer}$ between any two sprint intervals (repeated-measure ANOVA; $P=0.753$), indicating an equivalent and rapid elevation of $\dot{V}O_2$ in each condition. The excess oxygen consumed during exercise (EEOC, the volume consumed in excess of the resting rate) increased almost 14-fold as the exercise increased from 5 s to 60 s duration (0.17 µl O_2 $g^{-1}$to 2.2 µl O_2 g^{-1}, respectively, Table 1).

Oxygen consumption during the post-exercise period slowly declined towards resting rates during the next 90 min (Fig. 1). The recovery duration, defined as the time required for recovery $\dot{V}O_2$ to fall to within 2 standard errors of resting $\dot{V}O_2$, increased from 35 min following 5 s activity to 63 min following 60 s activity (Table 1). This relationship between activity duration and EPOC duration can be described by $y = 33.4 + 0.52x$ ($n=44$, $r^2=0.4578$), where y is EPOC duration (min) and *x* (sprint duration) ranges from 5 s to 60 s. An alternative definition of EPOC duration based upon the time required for recovery $\dot{V}O_2$ to decrease 95% towards the asymptote value predicted recovery from 5 s, 15 s, 30 s, and 60 s exercise to have required 29 min, 34 min, 50 min, and 57 min, respectively.

Sprint duration also had a significant and positive effect on the volume of oxygen consumed during recovery (EPOC volume, Table 1). EPOC volume equaled 0.13 ml O_2 g^{-1} following 5 s activity, and nearly doubled to 0.25 ml O_2 g^{-1} following 60 s of activity (Table 1). The pattern of metabolic recovery was similar following recovery irrespective of activity duration. A majority of the oxygen consumed by *D. dorsalis* following these short bursts of activity was consumed during the first 10 min of recovery. The absolute EPOC

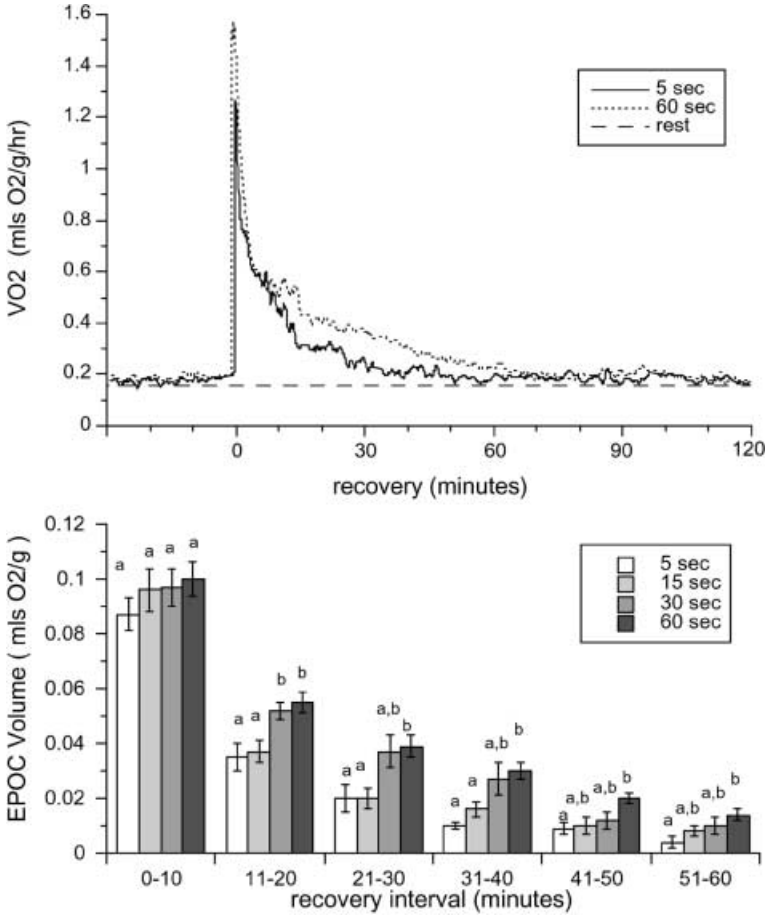

Fig. 1 Oxygen consumption and excess post-exercise oxygen consumption (EPOC) volumes resulting from maximal exercise of different durations. *Upper*: $\dot{V}O_2$ versus time for desert iguanas run 5 s (*solid line*) and 60 s (*broken line*). Each curve represents the mean of the same 11 animals. Data from animals run 15 s and 30 s were intermediate to these curves. *Lower*: An analysis of the pattern of recovery during the first 60 min of recovery. EPOC volumes are divided into six 10-min intervals for each of the four treatment groups. Means within a recovery interval with different superscripts are different

volume did not differ in the first 10 min of recovery as a function of sprint duration, although at later recovery periods the EPOC volume following 30 s and 60 s activity tended to be larger than following 5 s and 15 s sprints (Fig. 1).

Blood lactate concentrations following exercise

Resting blood lactate concentrations averaged 2.5 ± 0.7 mmol l^{-1}. Blood lactate increased by 6–20 mmol l^{-1} following 5–60 s burst activity. Post-exercise lactate concentrations, measured 5 min after the cessation of exercise, were correlated positively with exercise duration and were statistically different from each other (repeated measure ANOVA, $P < 0.0001$; Fig. 2). Statistically significant reductions in blood lactate concentrations during recovery occurred following all three exercise regimens (multiple paired-*t*, critical $P < 0.0253$), although energetically significant lactate removal ($\Delta LA > 5$ mmol l^{-1}) occurred only following 60 s of activity. Respiratory gas exchange during the recovery period was similar as that reported in Fig. 1 for uncannulated animals. EPOC duration, based on 95% recovery criteria, required 29 min, 27 min, and 83 min for 5 s, 15 s, and 60 s exercise, respectively.

Table 1 Summary of metabolic variables and distance traveled by *Dipsosaurus dorsalis* sprinting 5–60 s. Values are mean ± SEM; $n = 11$ (*EPOC* excess post-exercise oxygen consumption, *EEOC* excess oxygen consumed during exercise)

Sprint duration (s)	Exercise $\dot{V}O_2$ (ml O_2 g^{-1} h^{-1})	EEOC volume (ml O_2 g^{-1})	EPOC volume (ml O_2 g^{-1})	EPOC duration (min)	Approximate distance run (m)
5	1.3 ± 0.12	0.002 ± 0.0002^{a}	0.134 ± 0.0131^{a}	32.7 ± 2.10^{a}	3.5 ± 0.2^{a}
15	1.4 ± 0.15	0.005 ± 0.0006^{b}	0.156 ± 0.0205^{a}	43.0 ± 5.13^{a}	9.2 ± 0.7^{b}
30	1.4 ± 0.09	0.011 ± 0.0007^{c}	0.219 ± 0.018^{b}	52.1 ± 3.91^{b}	15.5 ± 0.8^{c}
60	1.5 ± 0.09	0.022 ± 0.0015^{d}	0.248 ± 0.0183^{b}	62.7 ± 2.48^{c}	27.3 ± 2.0^{d}
Repeated-measure ANOVA	$F = 0.40$	$F = 133.4$	$F = 11.32$	$F = 13.98$	$F = 81.0$
	$P = 0.753$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

[a, b, c, d] Different superscripts within columns indicate significantly different values (multiple paired-*t* with experiment-wise error = 5%, critical $P = 0.0085$)

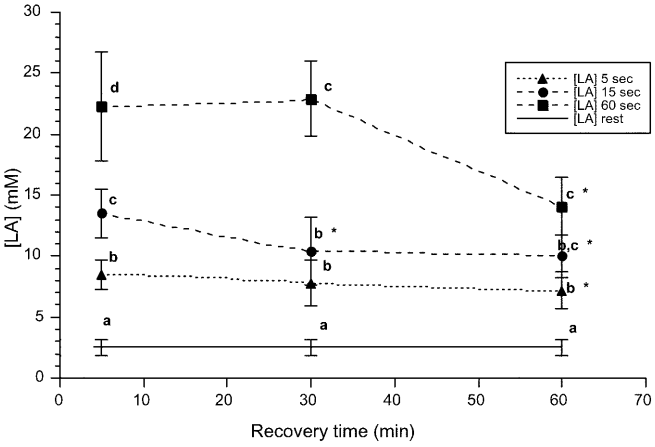

Fig. 2 Blood lactate concentrations ($n = 6$) during recovery following 5-s (*triangles*), 15-s (*circles*), and 60-s (*squares*) sprints. Values within a time period with different alphabetic superscripts are different (paired-*t*, $P < 0.0085$). An *asterisk* indicates values that are different from 5 min post-exercise values (multiple paired-*t*, critical $P < 0.0253$), indicating net lactate removal. Rates of oxygen consumption measured under the same recovery conditions were quantitatively similar to those illustrated in Fig. 1, and predicted EPOC durations of 27 min, 25 min, and 83 min, respectively

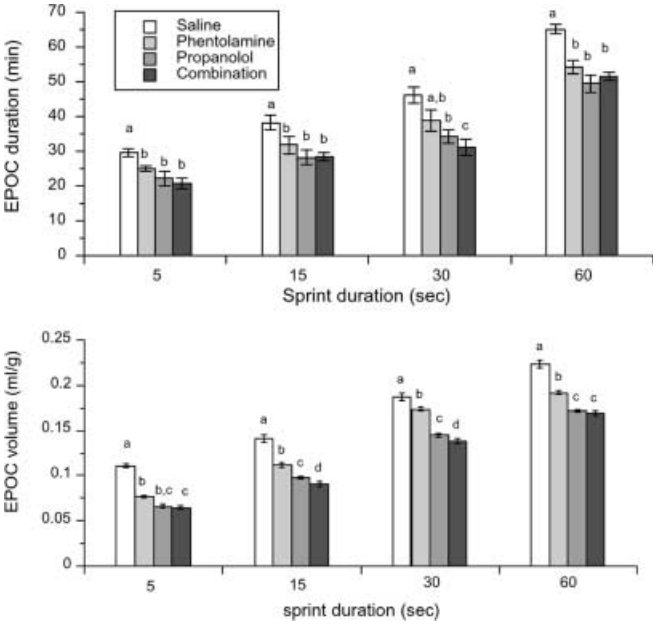

Fig. 3 The effect of saline, phentolamine, propranolol, and a combination of both drugs on EPOC duration (*upper*) and EPOC volume (*lower*) for all four exercise regimens. Low dosage treatments are shown. Values within sprint regimen with different superscripts are different ($n = 8$)

Adrenergic blockade effects on EPOC

EPOC duration was significantly shortened by injection of the α-blocker phentolamine, by the β-blocker propranolol, and when the two drugs were used in combination. At the lower dosage, EPOC duration was shortened by an average of 16% by phentolamine injection, 26% by propranolol injection, and by 27% when both drugs were used together (Fig. 3, upper). Although β blockade reduced recovery time more so than did α blockade, the three drug treatments had a statistically similar effect on reducing EPOC duration. In a single case (phentolamine injection followed by 30 s sprint) there was no reduction in EPOC duration, but in all other treatments both α and β blockade reduced the recovery time significantly. Increasing the dosage of each drug by a factor of ten yielded the same reduction in EPOC duration (data not shown).

A reduction in recovery time induced by adrenergic blockade results in a reduced EPOC volume if adrenergic blockade does not also depress metabolic rates of non-exercised animals. To test this possibility, animals at preferred body temperature were rested 2 h prior to saline or drug injection, and 2 or more hours after injection. Minimum rates of $\dot{V}O_2$ over 15 min were recorded both before and after injection, and these data are summarized in Table 2. Adrenergic blockade had no significant effect on the metabolic rate of non-exercised animals ($P = 0.15$). As a consequence, we conclude that the adrenergic blockade-induced reduction in EPOC duration also resulted in a reduction in EPOC volume (Fig. 3, lower). β-Blockade with propranolol, either alone or with phentolamine, had a slightly greater effect on reducing EPOC volume than did when α-blockade with phentolamine was tested alone (Fig. 3). When both drugs were used in combination, EPOC volume reduction ranged from 26% (after 60 s exercise) to 41% (after 5 s exercise) with a mean reduction over all four conditions of 31.8%.

Adrenergic blockade had no predictable effect on the volume of oxygen consumed during the first 10 min of recovery. There was no clear trend towards adrenergic reduction of the early EPOC volume, occurring in only two of four duration protocols. Adrenergic blockage did significantly alter the amount of oxygen consumed during the remaining 50 min recovery (Table 3). The most dramatic reduction in this phase of recovery occurred after 5 s of exercise, where phentolamine, propranolol, and both drugs in combination resulted in a 54–58% reduction from control values. Over all four exercise regimens, the volume of oxygen consumed during minutes 11–60 of recovery was reduced 39% by phentolamine injection, 49% by propranolol treatment, and 53% following injection of both drugs. In each case these reductions were statistically significant (Table 3).

Discussion

The metabolic and biochemical events associated with recovery from exercise have been under study in lizards and other ectotherms for several years (Gleeson 1996), but only recently have the behavioral determinants of recovery received similar attention. Body temperature has been known for some time to affect recovery rates of ectotherms (Bennett and Gleeson 1976; Gleeson 1980).

Table 2 Effect of catecholamine blockade on standard metabolic rate in *Dipsosaurus dorsalis*. Values are minimum $\dot{V}O_2$ (15-min average) during 2-h pre- and 2-h post-i.p. injection ($n = 6$; ml O_2 g^{-1} h^{-1}). Pre- and post-injection $\dot{V}O_2$ are not different

Treatment	Preinjection	Post-injection	Paired-*t*
Saline injection	0.22 ± 0.033	0.16 ± 0.020	$P = 0.06$
Drug injection[a]	0.22 ± 0.047	0.19 ± 0.040	$P = 0.15$

[a]14.3 μg g^{-1} propranolol plus 11.1 μg g^{-1} phentolamine in saline

Table 3 Effect of adrenergic blockade on the volume of oxygen consumed by *Dipsosaurus dorsalis* during early and late phases of recovery following maximal sprint activity of 5–60 s. Values are mean ± 1 SEM ($n = 8$; ml O_2 g^{-1}). Adrenergic blockade had no consistent effect on EPOC volumes during the first 10-min interval (only drug combination and saline shown). Dosages as in Table 2

Sprint duration (s)	EPOC volume (ml O_2 $g^{-1)}$					
	Early recovery (0–10 min)		Late recovery (11–60 min)			
	Saline	Combination	Saline	Phentolamine	Propanolol	Combination
5	0.052 ± 0.002^{a}	0.038 ± 0.002^{b}	0.059 ± 0.002^{a}	0.025 ± 0.002^{b}	0.026 ± 0.002^{b}	0.027 ± 0.002^{b}
15	0.062 ± 0.003^{a}	0.065 ± 0.002^{a}	0.079 ± 0.002^{a}	0.035 ± 0.002^{b}	$0.032 \pm 0.002^{b,c}$	0.026 ± 0.002^{c}
30	0.089 ± 0.003^{a}	0.090 ± 0.002^{a}	0.098 ± 0.002^{a}	0.082 ± 0.001^{b}	0.054 ± 0.002^{c}	0.048 ± 0.003^{c}
60	0.097 ± 0.002^{a}	0.092 ± 0.002^{b}	0.127 ± 0.003^{a}	0.093 ± 0.002^{b}	0.081 ± 0.002^{c}	0.077 ± 0.002^{bc}

[a,b,c] Values with different superscripts within the same row and interval comparison are different (experiment-wise error = 0.05, late recovery critical $P = 0.0085$)

Wagner and Gleeson (1996, 1997) have shown that behavioral selection of lower body temperatures occurs during recovery and that this reduction in temperature can affect recovery kinetics in the desert iguana. Other work in our laboratory has shown that exercise duration and exercise intensity can influence EPOC in lizards (T.V. Hancock et al., unpublished observations) although this does not appear to occur in mice (Baker and Gleeson 1998, 1999). Data reported in the present study are consistent with those of observations by T.V. Hancock et al. (unpublished) in that they show that activity of longer duration retards the rate and extends the endpoint of metabolic recovery of the desert iguana in a duration-dependent manner. The present study also demonstrates that a part of the metabolic response to brief activity appears to be mediated by the catecholamine elevations that were known to occur post-exercise (Gleeson et al. 1993), even when exercise durations are very short.

The effects of activity duration on EPOC

Combining data from this study with those from earlier studies of recovery in the desert iguana (Gleeson and Dalessio 1989; Wagner and Gleeson 1996; T.V. Hancock et al., unpublished observations) allow us to examine the duration dependency of recovery metabolism over a range of activity durations of 5–300 s. When these animals were subjected to maximal locomotor activity over those time scales, EPOC duration and volume were found to be duration dependent. This relationship between activity duration and EPOC is not a simple proportional relationship. EPOC duration roughly doubles as activity duration increases 12-fold from 5 s to 60 s (Table 1), but only increases another 50% as exercise duration increases from 60 s to 300 s (Wagner and Gleeson 1996). The data illustrate that the very act of vigorous running, however brief, will trigger a metabolic response that is expressed as elevated aerobic metabolism for a considerable period of time following the cessation of the behavior. The energetic expenditure of this post-exercise elevation of metabolism is many times the expense incurred during the locomotor act itself.

Our observations agree qualitatively with the results of sport and exercise physiologists interested in analogous questions in humans. Exercise duration has a positive effect on the duration and volume of excess post-exercise oxygen consumption in humans (Hagberg et al. 1980; Gore and Withers 1990a, 1990b; Bahr 1992). Studied activity durations in humans, however, are generally long (20–80 min) in length relative to the present study. Vigorous activity (at 125% $\dot{V}O_{2,max}$) of 60 s duration in humans similarly results in an EPOC response (Zanconato et al. 1991), and an early study of humans indicated that EPOC volume increased proportional to exercise duration over 1–3 min of exercise, but then achieved a steady value or decreased as exercise duration was extended through 6 min. A recent study of laboratory mice (Baker and Gleeson 1998) using a 5- to 60-s exercise protocol very similar to the present study concluded that over that range of exercise durations, EPOC duration and volume were fixed and independent of exercise duration. Other comparative data are not presently available. Our data suggest that there is a high cost to brief exercise in lizards, and that as animals locomote longer and cover greater distances, the additional cost due to EPOC becomes relatively small. This is confirmed by laboratory observations, and the meta-

bolic cost per unit distance run is shown to decline as activity duration is extended to longer and longer periods of time (T.V. Hancock et al., unpublished observations). However, at all activity durations, the substantial metabolic cost due to the post-activity EPOC causes an upward correction in estimates of cost per unit distance relative to traditional estimates of costs of transport.

Lactate and EPOC

While the evidence of a correlation between lactate accumulation and EPOC duration and volume is substantial, the evidence for a causal relationship in ectothermic vertebrates is poor. In the present study, lactate accumulation was proportional to activity duration (Fig. 2), which in turn is proportional to EPOC volume and duration. Levels of accumulation are consistent with other studies of this species (Gleeson 1985; Gleeson and Dalessio 1989; Wagner and Gleeson 1996).

We know that lactate removal post-exercise in this and other ectothermic vertebrates is dominated by gluconeogenic utilization of lactate to replenish muscle glycogen (Milligan and McDonald 1988; Withers et al. 1988; Gleeson and Dalessio 1989, 1990; Gleeson 1991, 1996; Fournier and Guderley 1992). This process requires metabolic energy that could be reflected in an elevated post-exercise oxygen consumption. These observations make a causal link between lactate and EPOC in ectotherms an attractive hypothesis, as it was to Margaria et al. (1933) and Hill and Lupton (1923) during the development of the since discarded O_2 debt concept during the first part of this century (see Gaesser and Brooks 1984). However, quantitatively significant amounts of lactate removal occur only following the 60-s activity period (Fig. 2), while EPOC occurs following both long and short activity durations. This suggests that EPOC volume is not obligatorily linked to the costs of lactate removal, as suggested in the earliest formulations of the O_2 debt concept.

Although a relationship between EPOC and the degree of lactate accumulation is well documented for humans and other animals (Bahr et al. 1992; Gleeson 1996), numerous studies have shown that the rate of lactate removal and EPOC duration can be decoupled. In rats and humans, lactate levels frequently return to resting levels before post-exercise oxygen consumption does (Bahr 1992; Bahr et al. 1992), and in ectothermic vertebrates the contrary is sometimes true, namely that lactate may remain elevated hours after post-exercise $\dot{V}O_2$ returns to normal (Bennett and Licht 1973; reviewed in Gleeson 1996). Studies where lactate levels were manipulated have shown that lactate and recovery $\dot{V}O_2$ are not causally related (Abramson et al.1927; Alpert and Root 1954; Roth et al. 1988). In *Dipsosaurus*, Wagner and Gleeson (1996) have shown that by lowering body temperature during recovery, EPOC duration can be shortened while lactate removal is simultaneously lengthened. Taken together, these observations suggest that the relationships between the rate of either lactate accumulation or removal and the magnitude of EPOC are not causal in nature, or at least are not the principle components determining EPOC.

Role of catecholamines in EPOC

Catecholamine stimulation, however, does appear to be a causative agent in determining EPOC volume and duration. Blockade of α- and β-adrenergic receptors had no appreciable effect on the magnitude of recovery oxygen consumption during the initial 10 min of recovery. In contrast, the remaining 50 min of excess oxygen consumption was reduced by 53% by α- and β-blockade over all exercise regimens (Table 3). These results suggest that the initial and later periods of EPOC have different physiological bases, a concept that has been promoted for many years (see review by Gaesser and Brooks 1984). Preliminary work in our laboratory indicates that rephosphorylation of ADP and creatine contribute to oxygen consumption in *Dipsosaurus* early in recovery (C.E. Crocker, personal communication), and we know that in other iguanid lizards both ATP and phosphocreatine levels are reduced following brief exercise (Bennett 1982). It is likely that the phase of catechol-insensitive recovery is due in part to this aspect of high-energy phosphate metabolism. Metabolic costs of elevated circulation, and resaturation of hemoglobin and myoglobin are also likely to contribute to this phase of recovery, based on analogy to mammalian systems (Bahr et al. 1992).

The evidence suggests that the latter period of elevated post-exercise oxygen consumption is partially ($\sim$50%) mediated by elevated catecholamines associated with exercise. Over the entire 60-min period, catecholamine-stimulated oxygen consumption may account for 25% of the total EPOC (after 60 s activity) to 41% of total EPOC (after 5 s of activity; Table 3). *Dipsosaurus dorsalis* exhibit elevated epinephrine and norepinephrine titers post-exercise (Gleeson et al. 1993), as do many other animals. Following 5 min of vigorous activity, desert iguanas demonstrate epinephrine and norepinephrine elevations of 5.8- and 10-fold, respectively, and epinephrine levels may stay elevated for 2 h or more. In various mammals, β-adrenergic blockade can reduce EPOC by approximately one-third (Barnard and Foss 1969; Cain 1971; Børsheim et al. 1994), and epinephrine supplementation can increase post-exercise recovery oxygen consumption (Gladden et al. 1982) and lactate oxidation (McDermott and Bonen 1992) in muscle. Catechol-stimulated oxygen consumption can persist hours after catecholamine levels have returned to normal in humans (Bahr et al. 1992). In *Dipsosaurus* we know that epinephrine stimulates glycogenic removal of lactate (Gleeson et al. 1993). In other lizard species and in alligators it also stimulates oxygen consumption of tissues and intact animals in a dose-dependent manner (Coulsen and Hernandez 1983; Gupta and Thapliyal 1985). Col-

lectively, these observations support a hypothesis that post-exercise elevations in catecholamines are responsible for a significant fraction of EPOC. The mechanism of this action is unknown in reptiles. In mammals it is thought to be related to epinephrine's effect on increasing cell membrane permeability to ions and consequent elevation in Na/K-ATPase activity, and to increased lipolysis and fatty acid cycling (Gaesser and Brooks 1984; Bahr et al. 1992). Elevated epinephrine also maintains an elevated post-exercise heart rate in humans (Bahr 1992), and stimulates gluconeogenic or glycogenolytic flux in numerous animals, including reptiles (Gleeson 1996), and these factors may also contribute to post-exercise $\dot{V}O_2$.

Implications

The present study illustrates that the EPOC resulting from brief periods of maximal exercise can account for 90% or more of the total oxygen consumption resulting from the locomotor behavior. The magnitude and duration of EPOC appears to be proportional to the duration of locomotor behavior when exercise intensity is maximal, at least over periods of locomotor behavior lasting 60 s or less. The duration of vigorous activity of many ectothermic vertebrates, including *Dipsosaurus*, is on this time scale (T.V. Hancock et al., unpublished observations), and hence the inclusion of EPOC in estimates in the costs of locomotor behavior will probably result in more accurate estimates of behavioral costs than if metabolic expenditure during the behavior alone is considered. The contribution of EPOC to the total energetic accounting of locomotor energetics suggests that traditional estimates of the costs of locomotion (Taylor 1977), which do not include EPOC costs, will severely under-estimate the true costs of brief locomotor behavior in *D. dorsalis*. Baker and Gleeson (1998) have shown that costs incurred post-exercise are the predominate contributor to overall estimates of activity costs in mice, and the present data and the data of Hancock et al. (unpublished observations) from this lizard species suggest that an analogous situation exist in ectothermic vertebrates also.

The utility of these laboratory observations to field behaviors does have limitation. The effects of catecholamine blockade suggest that a stress response may contribute as much as 50% to post-exercise metabolic costs. The hormonal response to vigorous exercise in the field may well be different than under laboratory treadmill conditions and thus the magnitude of EPOC following brief exercise in the field may be greater or less than those measured here, depending upon the natural stimulus of the behavior. Field measurements of plasma catecholamine changes following locomotor behavior of different types would resolve this question. Such data are presently unavailable.

In addition to possible variation in duration and variation in adrenal responses to activity, other variables (body temperature, locomotor intensity, locomotor frequency) play a role in altering the post-exercise metabolic response of lizards and other animals. Wagner and Gleeson (1996) have shown that EPOC can be reduced by voluntary reductions in body temperature post-exercise. Scholnick et al. (1997), Scholnik and Gleeson (2000) have presented data that prior activity can influence metabolic response to exhaustive exercise in lizards, and Houmard et al. (1991) and Weinstein and Full (1998) have shown that intermittent supramaximal exercise can be less fatiguing and alter post-exercise recovery than constant submaximal exercise at the same average work load. These behavioral variables need to be explored more fully before the energetic consequences of brief locomotor behavior in the field can be fully modeled.

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