Factors influencing the prediction of metabolic rate in a reptile

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Summary

1. Measurements of the rate of oxygen consumption ($\dot{V}O_2$) in the field are usually impractical, so several studies of endotherms have utilized heart rate ($f_H$) as a correlate of $\dot{V}O_2$ because of the tight relationship that often exists between the two variables. There have been several reports, however, where the relationship between $f_H$ and $\dot{V}O_2$ changes or disassociates under different physiological or psychological circumstances. This may be further confounded in ectothermic vertebrates, which experience relatively large fluctuations in body temperature ($T_b$).

2. The aim of the present study was to characterize in Rosenberg’s Goanna (Varanus rosenbergi) the relationship that exists between $T_b$, $f_H$ and $\dot{V}O_2$ at rest and at different levels of exercise, during periods of heating and cooling, and following ingestion of a meal.

3. The combinations of $T_b$ and $f_H$ were accurate at predicting $\dot{V}O_2$ of animals at different levels of exercise and recovery, and during the postprandial period.

4. Predictions of $\dot{V}O_2$ became less reliable during periods of relatively rapid heating when $f_H$ and blood flow increase for thermoregulatory purposes with no associated increase in $\dot{V}O_2$. To counter this, $f_H$ was excluded from the prediction equation when the rate of heating exceeded 20% of the predicted mass-dependent maximum attainable rate, and $\dot{V}O_2$ was predicted using $T_b$ alone.

5. The resultant $\dot{V}O_2$ prediction equation was used to estimate $\dot{V}O_2$ of seven animals that were allowed to thermoregulate behaviourally, and the mean predicted $\dot{V}O_2$ ($\dot{V}O_{2pred}$) was not significantly different from the mean measured $\dot{V}O_2$ ($\dot{V}O_{2max}$) for fasting or postprandial lizards.

Key-words: Body temperature, ectotherm, energy expenditure, heart rate, lizard, rate of oxygen consumption

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Introduction

Energy turnover and, in particular, how it is allocated to specific activities, is of central importance to the understanding of the physiological, behavioural and evolutionary ecology of organisms (McNamara & Houston 1996). Measurements of energy turnover through the determination of oxygen consumption rates ($\dot{V}O_2$) are usually impractical in the natural environment and, as a consequence, two main approaches have been developed to estimate the energetics of animals in the field (see Butler et al. 2004 for review).

One of these approaches is to utilize the association that exists between $\dot{V}O_2$ and heart rate ($f_H$). Heart rate may be readily telemetered or logged in wild animals, and shares a relationship with $\dot{V}O_2$ as described by the Fick equation for convection of oxygen through the cardiovascular system:

$$\dot{V}O_2 = f_H \times V_s \times (C_aO_2 - C_vO_2),$$

where $V_s$ is cardiac stroke volume (the product of $f_H$ and $V_s$ is cardiac output) and $(C_aO_2 - C_vO_2)$ is the difference in oxygen content between arterial ($C_aO_2$) and mixed venous ($C_vO_2$) blood. If oxygen pulse ($V_s \times (C_aO_2 - C_vO_2)$) remains constant, or varies in a systematic manner with $\dot{V}O_2$, then it should be possible to predict $\dot{V}O_2$ based on measurements of $f_H$.

For this to work, it is necessary to calibrate the relationship between $f_H$ and $\dot{V}O_2$, preferably using animals undergoing activities of interest and under similar environmental conditions to those in the wild. For example, Barnacle Geese and Bar-Headed Geese display a
markedly different $f_{\text{f}}/V_o_2$ relationship walking on a treadmill compared with flying in a wind tunnel (Ward et al. 2002). Furthermore, it has been shown in King Penguins that the relationship between $f_{\text{f}}$ and $V_o_2$ resulting from treadmill exercise is different from that when thermoregulating at low environmental temperatures (Froget et al. 2002). The cardio-metabolic consequences of digestion confuse the relationship between $f_{\text{f}}$ and $V_o_2$ in Steller Sea Lions, where postprandial increases in metabolism (i.e. specific dynamic action, SDA) are not associated with concomitant increases in $f_{\text{f}}$ (McPhee et al. 2003); implying that increases in $V_o_2$ and/or ($C_\text{O}_2 - C_\text{O}_2$) are more important than an increase in $f_{\text{f}}$ for circulatory oxygen transport during the digestive period. Despite possible changes occurring in the relationship between $f_{\text{f}}$ and $V_o_2$ with physiological or psychological state, several recent studies of endotherms have demonstrated that $f_{\text{f}}$ is an accurate correlate of the rate of energy expenditure (e.g. Bevan et al. 1995; Green et al. 2001; Ward et al. 2002).

Predictions of $V_o_2$ based on $f_{\text{f}}$ may be confused in ectothermic vertebrates because of large daily and seasonal fluctuations in body temperature ($T_b$). In this context, it has been reported for some species of fish that the effect of an increase in temperature is a right shift in the linear regression that describes the relationship between $f_{\text{f}}$ and $V_o_2$ during exercise (e.g. Claireaux et al. 1995; see Clark et al. 2005a and references within).

Few studies exist that determine the combined effects of activity and temperature on $f_{\text{f}}$ and $V_o_2$ in reptiles (Bennett 1972; Wilson 1974; Butler et al. 2002; Clark et al. 2005b). A study of marine iguanas exercising on a treadmill indicated that the $f_{\text{f}}/V_o_2$ regression line at a $T_b$ of 36°C was significantly right-shifted in comparison with that at a $T_b$ of 27°C and, consequently, the subsequent $V_o_2$ prediction equation incorporated a $Q_{10}$ function (Butler et al. 2002). This earlier study was limited to only two temperatures, and it is not known how accurately the equation predicts $V_o_2$ at temperatures other than those studied. A further complication in predicting $V_o_2$ from $f_{\text{f}}$ in reptiles could result from the thermally related hysteresis in $f_{\text{f}}$ that is known to exist in some species during heating and cooling (Bartholomew & Tucker 1963; Smith, Robertson & Davies 1978; Grigg & Seebacher 1999), where $f_{\text{f}}$ and blood flow are modified for thermoregulatory purposes without an accompanying change in $V_o_2$ (see Clark, Butler & Frappell 2005c).

Consequently, using the Fick equation to predict field $V_o_2$, particularly of ectotherms, requires a model that incorporates other variables in addition to $f_{\text{f}}$. The present study characterizes such a reptile, Rosenberg’s Goanna (Varanus rosenbergi), the relationship that exists between $T_b$, $f_{\text{f}}$ and $V_o_2$ at rest and at different levels of exercise, during periods of heating and cooling, and following ingestion of a meal. Thus, it was our aim to test the hypothesis that a combination of these biological factors could be utilized accurately to predict $V_o_2$ of animals under a controlled situation that simulated natural conditions. Rosenberg’s Goanna was chosen as it lives at a relatively high latitude and is therefore exposed to a broad range of $T_b$ values in the natural environment (10–38°C; see Christian & Weavers 1994; Rismiller & McKelvey 2000).

**Materials and methods**

**ANIMALS**

Lizards were obtained at the beginning of March from Kangaroo Island, South Australia, and kept in a temperature-controlled holding facility at La Trobe University at approximately 28°C for a maximum of 25 days before use in experiments. They were maintained on a 12:12 h light : dark photoperiod with access to a heat lamp during light hours. Animals had unlimited access to water and were fed twice weekly, though, where appropriate, they were fasted prior to experimentation (see below). All animals were returned to the holding facility following experimentation.

**RATE OF OXYGEN CONSUMPTION**

The rate of oxygen consumption was determined by placing a light-weight (approximately 4.5 g), transparent and loose-fitting mask over the head of the animal. The mask was fitted with an outlet tube through which air was drawn at a rate appropriate for the activity (1–8.1 min$^{-1}$), monitored by a calibrated mass flow meter (Sierra, model 810C Monterey, CA). A subsample of the air leaving the pump was passed through columns containing a drying agent (Drierite, Hammond, Xenia, Ohio, USA) and a carbon dioxide absorbent (Dragersorb, Lübeck, Germany) and analysed for the fractional content of oxygen by a gas analyser (model S-3A/F, Applied Electrochemistry, Pittsburgh, PA, USA). The rate of oxygen consumption was calculated from airflow through the mask and the difference between incoming and excurrent fractional concentrations of dry, carbon dioxide-free air (see appendix in Frappell et al. 1992). All values of $V_o_2$ are at standard temperature, pressure and dry (STPD) and expressed per kg.

**HEART RATE AND BODY TEMPERATURE**

Heart rate was obtained by attaching self-adhesive Ag/AgCl electrocardiogram (ECG) electrode pads and ECG leads to the dorsal surface of the animal, positioned such that they triangulated the heart. The ECG leads were connected to an amplifier (BIO amp, ADInstruments, Sydney, Australia). Body temperature was obtained by inserting a thermocouple 5–6 cm into the cloaca. All outputs were collected at 100 Hz (Powerlab 800, ADInstruments, Sydney, Australia) and displayed on a computer using Chart software (ADInstruments, Sydney, Australia).
PROTOCOL

The relationship between $T_b$, $f_H$ and $\dot{V}O_2$

Six adult animals (three males, three females; determined by morphological examination) of mean body mass ($M_b$) 1.55 ± 0.15 kg were fasted for at least 3 days then individually placed in a constant temperature room at the desired experimental temperature (range 14–36 °C) for at least 6 h prior to experimentation. Although $T_b$ was determined immediately before and after experimentation, $T_b$ values stated in the present report are an average of the two. Animals were then instrumented as described above and left to rest for at least 40 min on a variable-speed treadmill in the controlled temperature room until low, stable values of $f_H$ and $\dot{V}O_2$ were observed. They were then run on the treadmill at the maximum speed they could maintain for 5–10 min. The treadmill was ramped to maximum speed typically within 1 min; maximum speed varied with individual goannas and $T_b$ (range 0.3–2.9 km h$^{-1}$). When a lizard would no longer run while being enticed with gentle tapping on the hind legs, the treadmill was stopped and recordings continued for approximately 60 min during the recovery phase. All animals were studied on separate occasions at six or more $T_b$ values between 14 and 36 °C. Values (−1 min average of data points) were obtained from each animal 3–4 min prior to running, during the final 2 min of exercise, and at three stages during the recovery period while on the treadmill. Data from these experiments were used to establish an equation from which $\dot{V}O_2$ could be predicted from $f_H$ and $T_b$.

Validating the prediction equation

Raw data collected from *V. rosenbergi* used in a previous study (see Clark et al. 2005c) were used to validate the equation established for predicting $\dot{V}O_2$. In brief, seven animals were used both when fasted (>3 days fasted; fasted $M_b$ 1.35 ± 0.09 kg), and then 25 h after ingestion of a meal (meal size 10 ± 1% of fasted $M_b$, causes a significant increase in $f_H$ and $\dot{V}O_2$ approximately 25 h after ingestion, see Clark et al. 2005c). They were individually instrumented in a manner similar to that described above, and placed in a constant temperature room (floor area 1.5 × 2.0 m) at 14 °C until $T_b$ (monitored continuously) fell below 19 °C. At this point, a heat lamp positioned in one corner of the room was switched on. The lizards were free to move (all leads and tubes were suspended from a central point above the animal) and, on the majority of occasions, lizards positioned themselves under the lamp within an hour, otherwise they were moved by the experimenter. After heating, lizards were free to explore the room for 1–5 h, after which time the heat lamp was switched off and the lizards were allowed to cool again until $T_b$ fell below 19 °C. All data from resting and active animals were used to validate the equation established for predicting $\dot{V}O_2$.

DATA ANALYSIS AND STATISTICS

Repeated measures multiple linear regression was used to determine the relationship between $T_b$, $f_H$ and $\dot{V}O_2$. For the validation studies, data were averaged into 5-s blocks. Least-squares regressions were used where appropriate (see Results, Figs 4 and 5) to determine the relationships between variables. Repeated measures ANOVA was used to compare between measured and predicted values for animals freely allowed to thermoregulate (Table 1). Significance was considered at $P < 0.05$. Data are presented as mean ± SEM unless otherwise indicated. $N =$ number of animals, $n =$ number of data points.

Results

THE RELATIONSHIP BETWEEN $T_b$, $f_H$ AND $\dot{V}O_2$

The relationship at different $T_b$ values between $f_H$ and $\dot{V}O_2$ changed in a predictable fashion during periods of treadmill exercise and recovery (Fig. 1). The rate of oxygen consumption, as a function of $f_H$ and $T_b$, was best described by the following equation:

$$\log(\dot{V}O_2) = 1.738 \log(f_H) - 0.028T_b - 1.472,$$

where $r^2 = 0.80$, $P < 0.001$, $N = 6$, $n = 290$.

THE EFFECT OF FEEDING

The increase in $\dot{V}O_2$ that occurs following consumption of a meal (−10% of fasted $M_b$) has previously been characterized for *V. rosenbergi* (see Clark et al. 2005c) and, as it is primarily governed by $f_H$ and $T_b$, increases approximately 25 h after ingestion, owing to the heat increment of feeding, equation 2 reliably predicts changes in $\dot{V}O_2$ during the postprandial period (Fig. 2).

THE EFFECT OF HEATING AND COOLING

When given the opportunity to thermoregulate behaviourally, the majority of the lizards basked under the heat lamp to obtain a $T_b$ of 32–35 °C (e.g. Fig. 3a). Once the desired $T_b$ was reached, the lizards either moved away and cooled or shuttled in and out from underneath the heat lamp to maintain their preferred $T_b$ until the lamp was switched off.

During these periods, equation 2 tended to overestimate $\dot{V}O_2$ for all individuals, particularly during periods of relatively rapid radiant heating when the increase in $f_H$ was not matched by an increase in $\dot{V}O_2$ (Fig. 3b). The maximum rate at which a lizard can heat ($\Delta T_b/\Delta t$, °C min$^{-1}$) is negatively related to body mass for fasting and postprandial animals:

$$\log(\Delta T_b/\Delta t) = -0.823 \log(M_b) + 0.012,$$

where $r^2 = 0.80$, $P < 0.001$, $N = 6$, $n = 290$. 

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predicted values were calculated using equation 5. $\Delta$ is the difference between the predicted and measured $V_0$ expressed as a percentage.

*No significant difference between mean values of measured $V_0$ and predicted $V_0$ within a group ($P > 0.1$, repeated measures ANOVA).

Data for Animal 1 in the fasted state are illustrated in Fig. 3.

Fig. 1. The relationship between body temperature ($T_b$), heart rate ($f_H$) and the rate of oxygen consumption ($V_0$) for $V. rosenbergi$ at different levels of activity. Included are the relationships between $f_H$ and $V_0$ that occur during exercise and recovery at 15 °C, 22 °C, 29 °C and 36 °C (dashed lines), calculated using equation 2. $N = 6$, $n = 290$. Where $r^2 = 0.28$, $P < 0.05$, $N = 7$, $n = 14$. It was iteratively determined that equation 2 became inaccurate when the rate of heating exceeded 20% of the maximum mass-dependent attainable rate (this typically occurred during 70% of the initial heating period, and for approximately 15% of the entire experiment). As such, when an animal heated faster than 20% of the predicted maximum attainable rate for its body mass (calculated from equation 3), it was assumed that $f_H$ and $V_0$ had uncoupled for the purpose of thermoregulation. During these periods, $f_H$ was excluded from the prediction equation and $V_0$ was recalculated using only $T_b$ for resting animals when undergoing rapid heat exchange.
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(see equation 4; Fig. 4). This relationship between \( T_b \) and \( V_{O_2} \) is the same during heating and cooling within fasting and within postprandial lizards, but postprandial lizards have a higher \( V_{O_2} \) than fasting lizards as a result of SDA (Fig. 4). A common regression was adopted for the purpose of the \( V_{O_2} \) prediction equation:

\[
\log(V_{O_2}) = 0.022T_b - 0.132, \text{ eqn 4}
\]

where \( r^2 = 0.43, P < 0.001, N = 7, n = 68 \).

**Predicting \( V_{O_2} \)**

To summate, equation 2 reliably predicts \( V_{O_2} \) except during periods of relatively rapid heating when an uncoupling of \( f_H \) and \( V_{O_2} \) results in an overestimate of \( V_{O_2} \) (see Fig. 3b). This overestimation is corrected by incorporating equation 4 when \( T_b \) increases faster than 20% of the predicted maximum attainable rate (see Fig. 3c). The resultant equation can therefore be formalized as:

\[
\log(V_{O_2}) = C_1 \times \log(f_H) - C_2 \times T_b - C_3, \text{ eqn 5}
\]

where \( C_1 = 1.738, C_2 = 0.028 \) and \( C_3 = 1.472 \) when the mass-dependent rate of heating is less than 20% of the predicted maximum attainable value for the given body mass, and \( C_1 = 0.000, C_2 = -0.022 \) and \( C_3 = 0.132 \) when the rate of heating exceeds 20% of the maximum attainable value.

Subsequently, equation 5 was used to predict \( \tilde{V}_{O_2} \) of the seven individuals that were used in the validation study. Mean measured \( V_{O_2} \) (\( \tilde{V}_{O_2\text{meas}} \)) of these animals
DIGESTION

The act of processing, digesting and absorbing food is typically associated with an increase in the rate of oxygen consumption (i.e. SDA) which, in most vertebrates, is largely accompanied by an increase in $f_H$ (Kelbaek et al. 1987; Dumsday 1990; Wang, Burggren & Nobrega 1995; Hicks, Wang & Bennett 2000; Secor, Hicks & Bennett 2000; Wang et al. 2000, 2001; Clark et al. 2005c). However, in accordance with that occurring during exercise, the overall increase in systemic blood flow during digestion may not match the factorial increase in $\dot{V}_{O_2}$ (e.g. Hicks et al. 2000; Secor et al. 2000), thus an associated increase in $(C_{O_2} - C_{O_2})$ may also occur. It appears for $V. rosenbergi$ that the pattern of change in circulatory variables is such that the relationship between $f_H$ and $\dot{V}_{O_2}$ determined for exercising animals (equation 2) is maintained during digestion (Fig. 2).

The combined effects of digestion and exercise have been shown in some animals to elicit a response in $\dot{V}_{O_2}$ that is higher than either process could elicit on its own, yet the response is typically less than the sum of both processes combined (i.e. a partial ‘additivity’ of the responses; Segal & Gutin 1983; Secor et al. 2000; Bennett & Hicks 2001). From the data currently available, it appears that $f_H$ may be at least partly responsible for the cumulative effect in $\dot{V}_{O_2}$ that occurs during simultaneous digestion and exercise (e.g. Secor et al. 2000). Given that equation 5 did not significantly underestimate $\dot{V}_{O_2}$ of active postprandial $V. rosenbergi$ in the present study (Table 1 and Fig. 5), this ‘additivity’ of the responses should not be problematic when predicting energy expenditure of free-ranging, ectothermic vertebrates.

THERMOREGULATION

Of all vertebrates, reptiles may undergo the largest and most rapid daily fluctuations in $T_r$, thus contributing to the difficulty of predicting field metabolic rate of such animals. To account for the disassociation between $f_H$ and $\dot{V}_{O_2}$ that occurs in $V. rosenbergi$ during rapid heat exchange (see Introduction), the prediction of $\dot{V}_{O_2}$ in the present study required that $f_H$ be excluded from the prediction equation during periods of relatively rapid heating (>20% of maximum attainable rate). Clearly, operative temperatures in the natural environment will vary from those to which the animals were exposed in the present study, thus heat exchange will occur at different rates. It may be necessary in the wild therefore to monitor thermal conditions at ground level (e.g. radiant and ground temperatures), in
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combination with maximum attainable rates of heat exchange (determined from \( T_b \), trace on data loggers or transmitters; see below), to calculate at which point \( f_H \) is to be excluded from the \( V_o \) prediction equation. Correcting for this should not be difficult given that variations in operative temperature should influence only the intercept, but not the slope, of the relationship between \( M_b \) and the rate of heating (see equation 3).

As it is unlikely that the digestive state of an animal in the natural environment would be known, the incorporation of equation 4 into the prediction equation during relatively rapid heating assumes that all animals have a small postprandial increment in \( V_o \) during this period. Because such periods of rapid heating typically occur rather infrequently during the day for reptiles in the natural environment (see Christian & Weavers 1994; Rismiller & McKelvey 2000; Seebacher & Grigg 2001), it is unlikely that this assumption would produce a substantial error in a prediction of daily energy expenditure irrespective of the digestive state of the animal. In this context, if equation 4 is replaced either with the linear regression equation describing the relationship between \( V_o \) and \( T_b \) during the fasting period, or with that describing the relationship during the postprandial period (Fig. 4), the resultant mean \( \bar{V}_o \) values are not significantly different from the mean \( \bar{V}_o \) values (\( P > 0.1 \) in both cases).

**PREDICTING FIELD METABOLIC RATE**

With the advent and continued miniaturization of electronic data loggers/transmitters of \( f_H \) and \( T_b \) (e.g. Woakes, Butler & Bevan 1995), it is becoming increasingly possible to predict and monitor the energetics of animals in the natural environment (see Butler et al. 2004 and references within). The principal advantage of using this approach is that it can provide estimates of \( V_o \) at a far greater temporal resolution than many other methods (e.g. doubly labelled water, DLW); the resolution being limited only by the calibration procedure and the method of recording \( f_H \). The present study provides the most comprehensive evidence to date that it should be possible, based primarily on \( f_H \) and \( T_b \), to predict the energy expenditure of free-ranging ectotherms that undergo daily fluctuations in \( T_b \).

The suggested approach (i.e. equation 5) is accurate at predicting the mean \( \bar{V}_o \) of a group of animals, though the mean values hide the individual algebraic errors which ranged from -4.87% to +6.16% in fasting animals, and from -9.11% to +5.42% in postprandial animals (Table 1). Similar interindividual error ranges are typical when using a group prediction equation to estimate \( V_o \) of individual animals (e.g. Nolet et al. 1992; Bevan et al. 1994, 1995; Boyd et al. 1995; Hawkins et al. 2000; Froget et al. 2001; McPhee et al. 2003). It is generally accepted therefore that, although this method is capable of predicting \( V_o \) for specific activities in the field, it should be used only to estimate mean \( \bar{V}_o \) of a group of animals rather than estimating it for an individual animal (see Butler et al. 2004), unless specific animals are individually calibrated such that the prediction of energy expenditure of each animal is based on its own calibration equation. In either case, the error associated with the prediction of \( V_o \) must be taken into account (see Green et al. 2001; Butler et al. 2004).

Having established that there is a significant relationship between \( V_o \) and the variables of the prediction equation (e.g. \( f_H \) and \( T_b \)), it is important to remember that the relationship may not remain constant for individuals of a different physiological state. For example, it has been noted for King Penguins that the \( f_H \) relationship changes during the fasting period when animals spend long periods ashore (Froget et al. 2001; Fahlman et al. 2004). There are data for lizards that suggest seasonal changes in \( V_o \) (Christian & Conley 1994; Christian, Bedford & Schultz 1999; de Souza et al. 2004), though it has not yet been ascertained whether \( f_H \) follows a similar seasonal pattern. Nevertheless, if our approach is to be used to predict field \( V_o \) of *V. rosenbergi* throughout the entire year, it will be necessary to clarify the relationship between \( T_b \), \( f_H \) and \( V_o \) on wild-caught animals at different times of the year, and potentially incorporate a seasonal component into the resultant equation.

The present study details the principles involved when predicting metabolic rate of a thermoregulating ectotherm, and the findings suggest that it is possible to use primarily \( f_H \) and \( T_b \) to predict the energetics of free-ranging ectothermic vertebrates in the natural environment. The interest in using \( f_H \) to estimate field \( V_o \) of birds and mammals has increased in recent years, and the principles outlined in the present study should encourage similar studies on energetics of free-ranging ectothermic vertebrates.

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**References**


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