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Review

# Effects of temperature and hypercapnia on ventilation and breathing pattern in the lizard *Uromastyx aegyptius microlepis*☆

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**Abstract**

In most reptiles, the ventilatory response to hypercapnia consists of large increases in tidal volume ($V_T$), whereas the effects on breathing frequency ($f_R$) are more variable. The increased $V_T$ seems to arise from direct inhibition of pulmonary stretch receptors. Most reptiles also exhibit a transitory increase in ventilation upon removal of $CO_2$ and this post-hypercapnic hyperpnea may consist of changes in both $V_T$ and $f_R$. While it is well established that increased body temperature augments the ventilatory response to hypercapnia, the effects of temperature on the post-hypercapnic hyperpnea is less described. In the present study, we characterise the ventilatory response of the agamid lizard *Uromastyx aegyptius* to hypercapnia and upon the return to air at 25 and 35 °C. At both temperatures, hypercapnia caused large increases in $V_T$ and small reductions in $f_R$, that were most pronounced at the higher temperature. The post-hypercapnic hyperpnea, which mainly consisted of increased $f_R$, was numerically larger at 35 compared to 25 °C. However, when expressed as a proportion of the levels of ventilation reached during steady-state hypercapnia, the post-hypercapnic hyperpnea was largest at 25 °C. Some individuals exhibited buccal pumping where each expiratory thoracic breath was followed by numerous small forced inhalations caused by contractions of the buccal cavity. This breathing pattern was most pronounced during severe hypercapnia and particularly evident during the post-hypercapnic hyperpnea. © 2002 Published by Elsevier Science Inc.

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## 1. Introduction

Reptiles exhibit marked ventilatory responses to elevated levels of $CO_2$ in the inspired air that have been characterised in numerous species belonging to most major groups of Reptilia. In general, tidal volume ($V_T$) increases progressively during hypercapnia whereas the effects on breathing frequency ($f_R$) are more variable. In fact, some animals exhibit a decrease in $f_R$ while others exhibit an increase, leading to variable changes in overall ventilation ($\dot{V}_e$). The ventilatory responses appear to depend on the level of $CO_2$ and most animals increase $\dot{V}_e$ during low levels of hypercapnia, whereas higher levels generally lead to a depression. The effects on $V_T$ are predominantly caused by direct inhibition of pulmonary stretch receptors

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(PSRs) by $CO_2$, which leads to diminution of the Hering–Breuer reflex. The effects on $f_R$ appear to result from several opposing effects of $CO_2$ on different receptor groups. Thus, while $CO_2$ stimulates ventilation through a direct effect on central chemoreceptors within the medulla (Hitzig and Jackson, 1978; Branco and Wood, 1993) and vascular receptors on the major arteries (Wang et al., 1998), it also exerts inhibitory actions through upper airway chemoreceptors and intrapulmonary chemoreceptors (IPCs) (Milsom, 1995a,b).

The steady-state responses to hypercapnia differ among species, but virtually all species exhibit a marked increase in ventilation following the removal of $CO_2$ (termed the post-hypercapnic hyperpnea), which reflects the withdrawal of inhibitory receptor feed-back from IPCs and those in the upper airways (Milsom, 1995a,b). This post-hypercapnic hyperpnea has been documented in snakes, lizards and alligators where it is characterised by an immediate increase in $f_R$ while $V_T$ normally decreases (Randall et al., 1944; Nielsen, 1961; Templeton and Dawson, 1963; Glass and Johansen, 1976; Nolan and Frankel, 1982; Alligators—S.J. Warburton and T. Wang, unpublished).

It is well established that ventilatory responses to hypoxia and hypercapnia are greatly enhanced with elevated body temperature. Thus, at increased temperatures the threshold $CO_2$ concentration at which ventilatory responses occur decreases, and larger ventilatory changes occur on exposure to a given level of $CO_2$ (Glass and Wood, 1983; Dupré et al., 1989). In contrast, the only study regarding the effects of temperature on the post-hypercapnic hyperpnea indicated that this response decreased with increased temperature in the snake *Coluber constrictor* (Nolan and Frankel, 1982).

*Uromastyx* is a large herbivorous lizard that inhabits dry areas in the Middle East. This species undergoes large diurnal changes in body temperature in its natural environment and has been shown to increase $V_T$ and $\dot{V}_e$ during hypercapnia and with increased temperature (Von Saalfeld, 1934a). The post-hypercapnic hyperpnea has, however, not been reported. Both Von Saalfeld (1934a,b) and a more recent study (Al Ghamdi et al., 2001) have shown that this species employs gular pumping to aid lung inflation when tidal volume is increased. This observation does not agree with the common view that Squamate reptiles generally employ intercostal muscles for the generation of ventilatory airflows (e.g. Bellaires, 1970; but see Brainerd, 1999). It is possible that buccal breathing would be particularly pronounced when respiratory drive is elevated. Indeed, Von Saalfeld (1934a,b) mentioned that the number of buccal breaths increases with elevated temperature in *Uromastyx*.

In the present study, we aimed to characterise the effects of temperature on the ventilatory response to inspired hypercapnia with a particular focus on the post-hypercapnic hyperpnea. Because of the high ventilatory activity, we were also interested in the possibility that gular pumping occurred and contributes significantly during the post-hypercapnic hyperpnea of non-anaesthetised and undisturbed lizards.

## 2. Material and methods

### 2.1. Animals

Experiments were conducted on five adult specimens of *Uromastyx aegyptius microlepis* of either sex with body mass varying from 430 to 1160 g. These lizards were collected in Saudi Arabia and had been kept at the University of Birmingham (Birmingham, England) for several years on a diet of vegetables and fruit (details of animal husbandry are given elsewhere; Gardner et al., 1993; Al Ghamdi et al., 2001).

### 2.2. Ventilation measurements

Ventilation was measured using a mask as described by Glass et al. (1978) and modified by Wang and Warburton (1995). An individual plastic mask was constructed for each lizard, which covered the lizard's head completely, while minimising dead space. A rubber collar provided a gas-tight seal around the animal's neck. The mask received a continuous flow of air or gas mixtures at approximately 1000 ml $min^{-1}$ and, because the inflow of air was constant, changes in flow at the outlet were indicative of ventilatory flows. A resistor (Fleisch tube) was inserted into the outflow of the mask and pressure changes across this resistance were measured with a Valedyne (MP-45-1-871) differential pressure transducer. The output of the transducer was collected at 50 $s^{-1}$ using a computerised data-acquisition system (Biopac Systems MP100). All masks were calibrated individually by injection of known air volumes using a syringe. Because *Uromastyx* exhibits long

apnoeic periods, the calibration could be performed during the non-ventilatory periods while the animals were wearing the mask. The relationship between integrated area of the pressure transducer signals and volume of air injected into the mask was accurately described by a linear regression ($r^2 > 0.9$; Fit S.E. $< 0.085$, in all cases) whose equation was used to calculate tidal volume ($V_T$). The active expiration, which started a ventilatory cycle was used to determine $V_T$ and breathing frequency ($f_R$). Total expired ventilation ($\dot{V}_e$) was calculated as the product of $V_T$ and $f_R$.

### 2.3. Experimental protocol

The mask was attached to the lizard's head at least 24 h before experimentation, to allow the animal to recover from the disturbance of handling and to become accustomed to wearing the mask. Each animal was placed in an experimental chamber ($50 \times 30 \times 20$ cm) held at the experimental temperature, where they remained undisturbed until the following day. The experimental temperatures (25 and 35 °C) were attained by keeping the lizards inside a climatic chamber (Thermatek). Three lizards were measured at 25 °C and then at 35 °C, whereas this order was reversed in the other two individuals.

An initial, stable ventilatory pattern was recorded for a minimum of 30 min from all animals breathing room air. $CO_2$ was then added to the stream of gases supplying the mask. The hypercapnic exposures (1, 3, 5 and 7% $CO_2$ in air) were applied in random order and each lasted for 30 min. The animals were returned to room air after each hypercapnic exposure and a new level of $CO_2$ was then applied after the breathing pattern had returned to the initial level (this normally occurred within 10–20 min).

### 2.4. Data handling and statistical analysis

At each level of $CO_2$, we analysed a 5-min period, which was chosen to include the most regular breathing pattern recorded during the exposure. This breathing pattern was assumed to represent the steady-state ventilatory response at any given level of hypercapnia. To analyse the ventilatory response upon return to air, $f_R$, $V_T$ and $\dot{V}_e$ were measured over the following time intervals: 0–1 min, 1–2 min, 2–4 min, 4–6 min and finally at 18–22 min, after switching from the hypercapnic gas mixtures to air.

Comparisons of ventilatory variables between experimental temperatures were made using a paired Student's *t*-test. The effects of $CO_2$ level on the ventilatory parameters were tested using an one-way repeated measures ANOVA followed, whenever necessary, by a posteriori multiple comparison procedure (Dunnett's test). When necessary to apply the premises of normality and homoscesdascity, the data were log transformed. Differences were considered statistically significant at a 95% level of confidence ($P \leq 0.05$). All data are presented as mean $\pm$ 1 S.E.M.

## 3. Results

### 3.1. Steady-state ventilatory response to hypercapnia

The steady-state breathing patterns of one individual specimen of *Uromastyx,* exposed to increasing levels of $CO_2$, are depicted in Fig. 1, while the ventilatory parameters of all five specimens are listed in Table 1. As shown in Fig. 1, high levels of $CO_2$ were often associated with changes in the patterns of individual breaths. When breathing room air or 1% $CO_2$ each breath consisted of a single cycle of aspiratory, costal breathing, that started in a passive expiration, a pattern typical of lizards (e.g. Von Saalfeld, 1934a; Carrier, 1987; Al Ghamdi et al., 2001). When breathing air plus 3% or 5% $CO_2$ each breath ended in a sharp inspiration and when breathing 7% $CO_2$ each breath ended with several (4–7) similar inspiratory flows. This pattern is consistent with the onset of gular pumping, as previously described for *Uromastyx* (Von Saalfeld, 1934a; Al Ghamdi et al., 2001). Gular pumping was rarely displayed when the animals were breathing room air (only one individual), but occurred more frequently when $CO_2$ was higher than 5% and particularly when combined with increased temperature (Table 2).

The mean values for the steady-state response for all animals during hypercapnia are presented in Fig. 2, which also includes proportional changes in ventilatory parameters. Increased temperature was associated with a small but significant reduction of $V_T$ during air exposure (Fig. 2a). At both temperatures, all animals exhibited similar large increases in $V_T$ during hypercapnia (Fig. 2a) though the proportional change at 7% $CO_2$ was

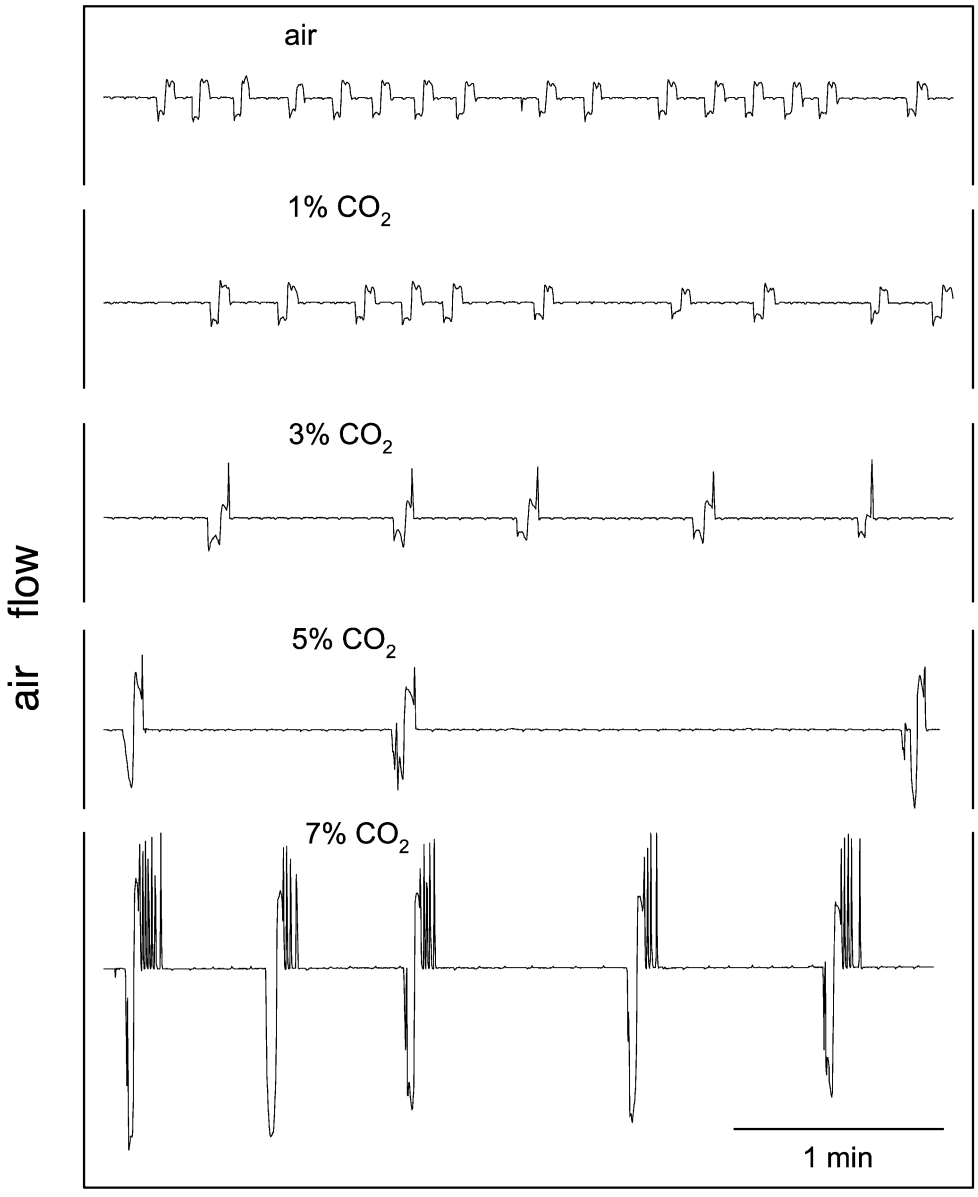


Fig. 1. Representative breathing traces from an individual *Uromastyx aegyptius* at 25 °C exposed to different levels of $CO_2$. Progressive hypercapnia, in general, caused tidal volume ($V_T$) to increase and breathing frequency ($f_R$) to decrease. At 7% $CO_2$ the repeated rapid inspiratory flows following each costal ventilatory cycle indicate the use of gular pumping (see Section 4). The unit of airflow is arbitrary and the same for all conditions.

larger at 35 °C (Fig. 2d). Breathing frequency increased significantly with increased temperature, and this difference in $f_R$ persisted throughout all hypercapnic exposures (Fig. 2b). Hypercapnia elicited a significant reduction in $f_R$ at both temperatures (Fig. 2c). The proportional decrease in $f_R$ was smaller than the increase in $V_T$, which resulted in an elevation of $\dot{V}_e$ during hypercapnia. This effect was most pronounced at 35 °C, where the increase in $\dot{V}_e$ during progressive hypercapnia was statistically significant (Fig. 2f).

### *3.2. The post-hypercapnic hyperpnea*

Figure 3 shows the ventilatory pattern immediately after the inspiratory gas was changed from

Table 1
Steady-state values of the ventilatory parameters of *Uromastyx aegyptius* exposed to different levels of $CO_2$, at 25 °C and 35 °C

| | 25 °C | | | 35 °C | | |
|---|---|---|---|---|---|---|
| | $f_R$ ($min^{-1}$) | $V_T$ (ml $kg^{-1}$) | $\dot{V}_e$ (ml $kg^{-1}$ $min^{-1}$) | $f_R$ ($min^{-1}$) | $V_T$ (ml $kg^{-1}$) | $\dot{V}_e$ (ml $kg^{-1}$ $min^{-1}$) |
| Air | 3.2±1.1 (1.6–4.3) | 8.0±2.5 (4.2–10.7) | 24.5±9.4 (17.4–36.7) | 7.7±2.1 (4.2–9.8) | 4.4±1.1 (2.6–5.3) | 33.3±11.7 (20.3–43.8) |
| 1% $CO_2$ | 2.2±1.0 (0.7–3.5) | 9.5±3.3 (6.0–14.7) | 18.5±5.4 (9.9–20.8) | 8.1±2.3 (4.2–10.2) | 5.2±1.1 (3.4–6) | 41.0±14.2 (25–61.4) |
| 3% $CO_2$ | 1.6±1.0 (0.8–3.3) | 14.2±4.1 (8.0–18.2) | 23.6±18.8 (9.1–54.8) | 7.0±1.6 (5.2–8.6) | 10.7±2.8 (6.3–13.8) | 74.2±27.4 (52.9–117.3) |
| 5% $CO_2$ | 1.6±0.7 (0.6–2.4) | 23.9±17.6 (9.4–53.7) | 41.4±49.0 (11.7–128.5) | 6.2±2.7 (2.7–9.6) | 14.7±9.1 (3.9–27.7) | 87.0±79.3 (37.4–226) |
| 7% $CO_2$ | 1.7±0.7 (1.0–2.8) | 30.6±20.1 (7.8–52.4) | 56.1±50.2 (12.6–132.7) | 4.9±1.9 (2.9–7.3) | 30.0±8.8 (16.7–37.4) | 142.0±57.1 (85.8–231.8) |

Values are mean±1 S.E.M., while minimal and maximal values are listed in parentheses, $N=5$.

Table 2
Numbers of individuals using gular pumping ($N_{GP}$) and frequency of gular breaths per minute ($f_{GB}$) in *Uromastyx aegyptius* during steady-state exposure and during different intervals of the post-hypercapnic hyperpnea

| | 25 °C | | 35 °C | |
|---|---|---|---|---|
| | $N_{GP}$ | $f_{GB}$ | $N_{GP}$ | $f_{GB}$ |
| Air | 1 | 1.64±3.7 (0–8.2) | 0 | 0±0 (0–0) |
| 1% $CO_2$ | 2 | 2.0±4.4 (0–9.8) | 0 | 0±0 (0–0) |
| 30 s | 1 | 7.0±15.7 (0–35.0) | 0 | 0±0 (0–0) |
| 90 s | 1 | 7.2±16.1 (0–36.0) | 0 | 0±0 (0–0) |
| 180 s | 1 | 4.5±10.1 (0–22.5) | 0 | 0±0 (0–0) |
| 300 s | 1 | 4.6±10.3 (0–23) | 0 | 0±0 (0–0) |
| 1200 s | 1 | 1.6±3.2 (0–6.3) | 0 | 0±0 (0–0) |
| 3% $CO_2$ | 4 | 0.84±0.7 (0–1.7) | 2 | 0.9±1.4 (0–3.2) |
| 30 s | 2 | 12.0±21.2 (0–49.0) | 2 | 1.6±2.2 (0–4.0) |
| 90 s | 2 | 11.4±19.5 (0–45.0) | 0 | 0±0 (0–0) |
| 180 s | 2 | 4.9±10.4 (0–23.5) | 0 | 0±0 (0–0) |
| 300 s | 1 | 4.1±9.2 (0–20.5) | 2 | 1.3±2.0 (0–4.5) |
| 1200 s | 0 | 0±0 (0–0) | 0 | 0±0 (0–0) |
| 5% $CO_2$ | 1 | 0.5±0.4 (0–1.0) | 4 | 1.5±1.8 (0–3.6) |
| 30 s | 3 | 23.4±34.7 (0–79.0) | 3 | 21.4±28.9 (0–56.0) |
| 90 s | 3 | 6.4±8.6 (0–20.0) | 2 | 11.6±16.3 (0–34.0) |
| 180 s | 4 | 4.5±5.1 (0–10.5) | 2 | 11.3±15.5 (0–30) |
| 300 s | 1 | 0.4±0.9 (0–2.0) | 2 | 9.6±14.3 (0–32) |
| 1200 s | 1 | 0.01±0.04 (0–0.8) | 0 | 0±0 (0–0) |
| 7% $CO_2$ | 1 | 1.0±1.7 (0–4.0) | 4 | 2.6±2.0 (0–4.4) |
| 30 s | 5 | 27.8±15.0 (13.0–53.0) | 3 | 21.8±28.3 (0–57.0) |
| 90 s | 4 | 14.6±12.3 (0–34.0) | 2 | 11.8±16.3 (0–33.0) |
| 180 s | 3 | 10.7±14.1 (0–34.5) | 2 | 10.1±14.6 (0–32) |
| 300 s | 2 | 5.3±10.5 (0–24.0) | 2 | 11.2±16.5 (0–36.5) |
| 1200 s | 1 | 1.7±3.7 (0–8.3) | 1 | 0±0 (0–0) |

Values are mean±1 S.E.M., while minimal and maximal values are listed in parentheses, $N=5$.

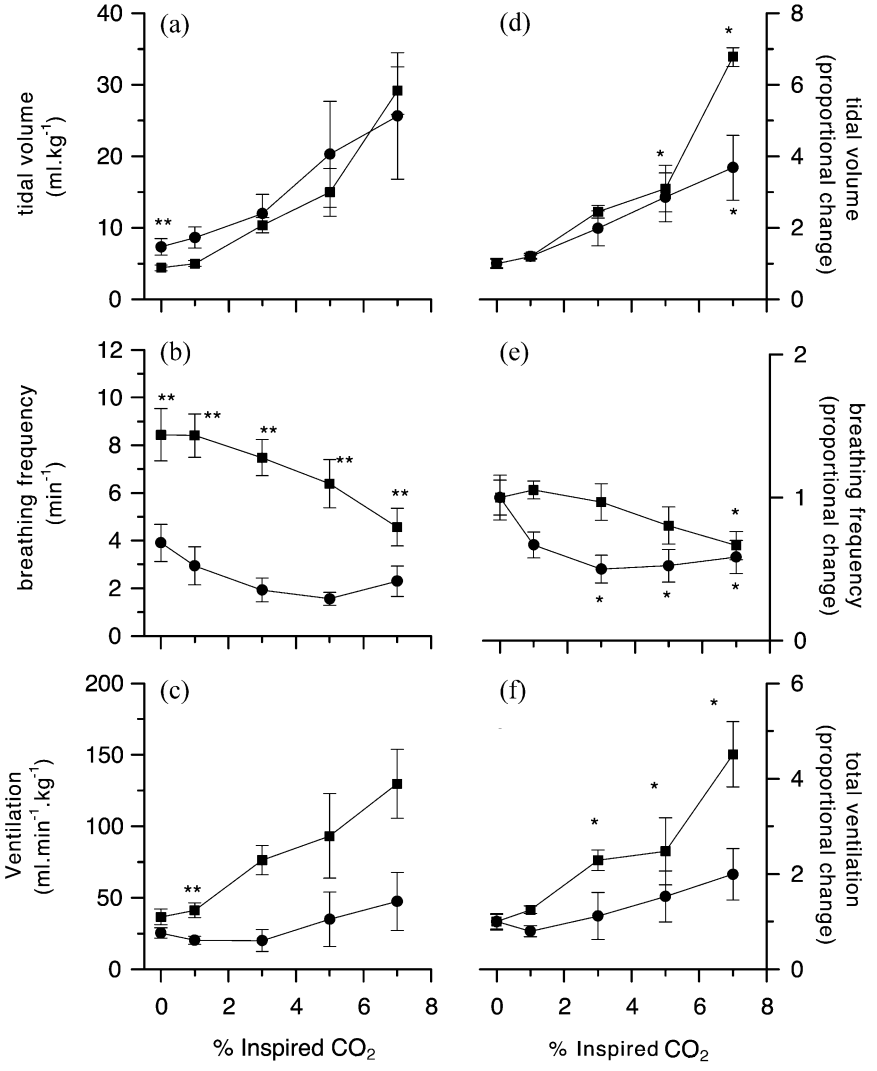


Fig. 2. The effects of hypercapnia on tidal volume ($V_T$), breathing frequency ($f_R$) and total ventilation ($\dot{V}_e$) in *Uromastyx aegyptius* at 25 °C (●) and 35 °C (■), during steady-state exposure to hypercapnia. The right hand panels depict proportional changes in the reported parameters relative to the values recorded during the exposure to air (taken as unity). An asterisk indicates values that are significantly different from the value obtained during normocapnia; double asterisk indicate significant differences between temperatures. Values are expressed as means ± 1 S.E.M. ($N=5$).

7% $CO_2$ to room air (i.e. the post-hypercapnic hyperpnea) in one individual at both experimental temperatures. Removal of $CO_2$ elicited an immediate increase in $f_R$ and changes in breathing pattern that consisted of an immediate and pronounced use of gular pumping. A similar pattern occurred in most of the animals studied, although some individuals did not exhibit gular pumping even during the post-hypercapnic hyperpnea (Table 2).

The temporal changes in ventilatory parameters following the different levels of hypercapnia are presented in Figs. 4 and 5. Breathing frequency always increased during the post-hypercapnic hyperpnea (Fig. 4b,e), but this increase was more pronounced at 25 °C (Fig. 5b,e). The changes in $V_T$ during the post-hypercapnic hyperpnea varied between the two temperatures. Thus, at 25 °C, $V_T$ remained high during the initial post-hypercapnic hyperpnea, whereas $V_T$ decreased immediately fol-

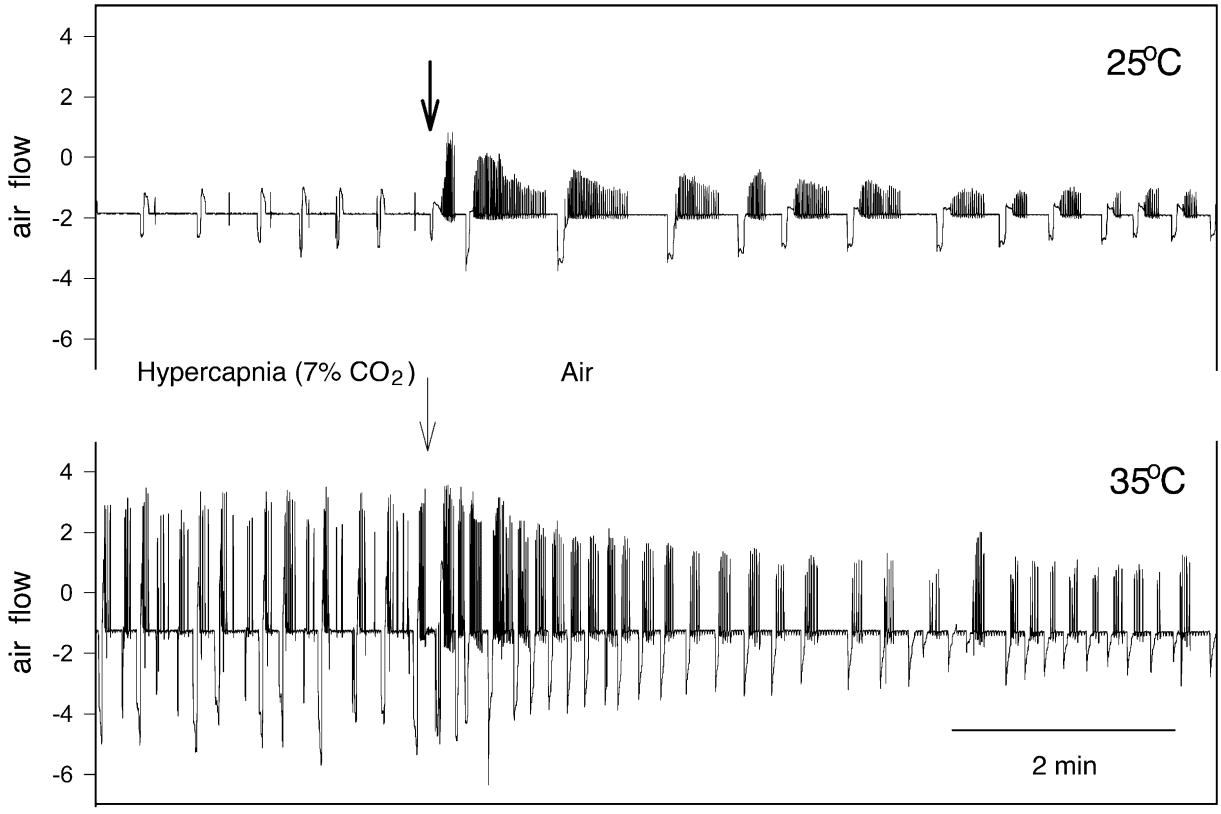


Fig. 3. Representative breathing traces of one individual *Uromastyx aegyptius* at 25 and 35 °C upon the return to air after being exposed to 7% $CO_2$ (the transition from hypercapnia to air is indicated by arrows). Note that there is an initial increase in tidal volume ($V_T$) at 25 °C, while $V_T$ decreases and breathing frequency ($f_R$) increases at 35 °C. At both temperatures, the contribution of gular pumping to lung ventilation increases in following the return to air. The units of the airflow are arbitrary and the same for both conditions.

lowing the return to room air at 35 °C. These changes in $V_T$ and $f_R$ resulted in an immediate transient increase in $\dot{V}_e$ at both temperatures (Fig. 4c,f). However, the proportional increase in $\dot{V}_e$ varied between temperatures, being relatively large (2–5-fold) at 25 °C but barely perceptible at 35 °C (Fig. 5c,f).

The effects of the preceding $CO_2$ levels on the immediate post-hypercapnic hyperpnea are depicted in Fig. 6. This figure shows the steady state response recorded during hypercapnia (filled symbols) and the post-hypercapnic hyperpnea recorded during the first minute after switching the inspiratory gas to room air (open symbols). This representation shows that the immediate increase in $\dot{V}_e$ during the post-hypercapnic hyperpnea, which was significant following exposure to 3% $CO_2$ at both temperatures, (Fig. 6c,f) is caused by significant increases in $f_R$ (Fig. 6b,e), whereas $V_T$ remained nearly unchanged (Fig. 6a,d).

## 4. Discussion

### *4.1. Steady-state ventilatory response to hypercapnia*

The present study on *Uromastyx* shows a large increase in $V_T$ during progressive hypercapnia. The increased $V_T$ during hypercapnia is consistent with previous observations on *Uromastyx* (Von Saalfeld, 1934a) and most available data on other reptiles (Nielsen, 1961; Templeton and Dawson, 1963; Glass and Johansen, 1976; Nolan and Frankel, 1982; Glass and Wood, 1983; Abe and Johansen, 1987; Wang and Warburton, 1995; Wang et al., 1998; Gratz, 1979; Glass et al., 1979). This response is normally attributed to a direct and

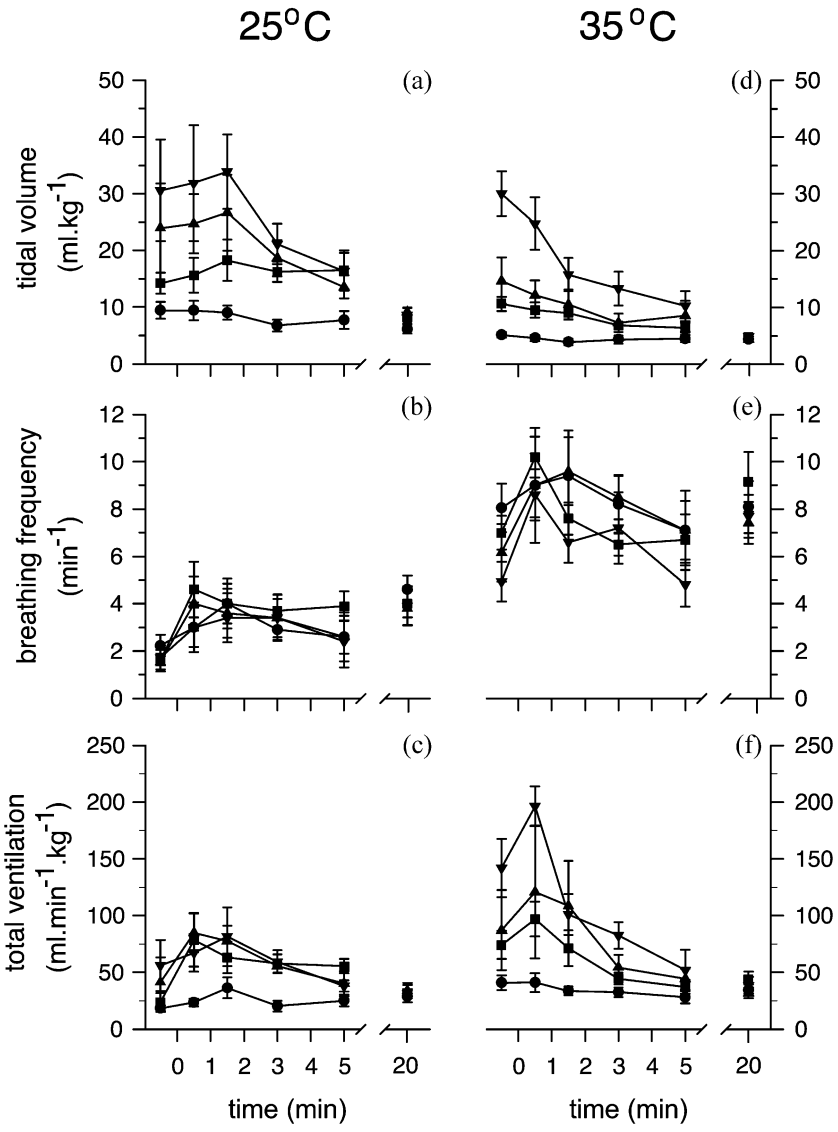


Fig. 4. The temporal development of the post-hypercapnic hyperpnea in *Uromastyx aegyptius* at 25 (●) and 35 °C (■). The figures include values for tidal volume ($V_T$), breathing frequency ($f_R$) and total ventilation ($\dot{V}_e$) during steady-state exposure to hypercapnia. Inhalation of air following the hypercapnic exposure commenced at 0 min (inspired $CO_2$ 1%=●; 3%=■; 5%=▲; and 7%=▼). Values are presented as means±1 S.E.M. ($N$=5).

inhibitory role of $CO_2$ on the pulmonary stretch receptors (PSRs), so that hypercapnia inhibits the Hering–Breuer reflex and thereby reduces the inspiratory breaking that normally follows increased lung volume (Milsom, 1995b). Thus, neural recordings from PSRs show that $CO_2$ reduces or even abolishes the response to lung inflation (Jones and Milsom, 1982; Powell et al., 1988; Douse et al., 1989; Sundin et al., 2001). However, the response to sudden lung inflation in *Uromastyx*, which caused immediate cessation of buccal breathing, was unaffected by addition of high levels of $CO_2$, indicating that their lung stretch receptors are insensitive to $CO_2$ (Al Ghamdi et al., 2001). The changes in $V_T$ during hypercapnia where not influenced by temperature. These observations are consistent with the apparently low temperature sensitivity of pulmonary stretch receptors in reptiles (Furilla and Bartlett, 1988; Douse et al., 1989).

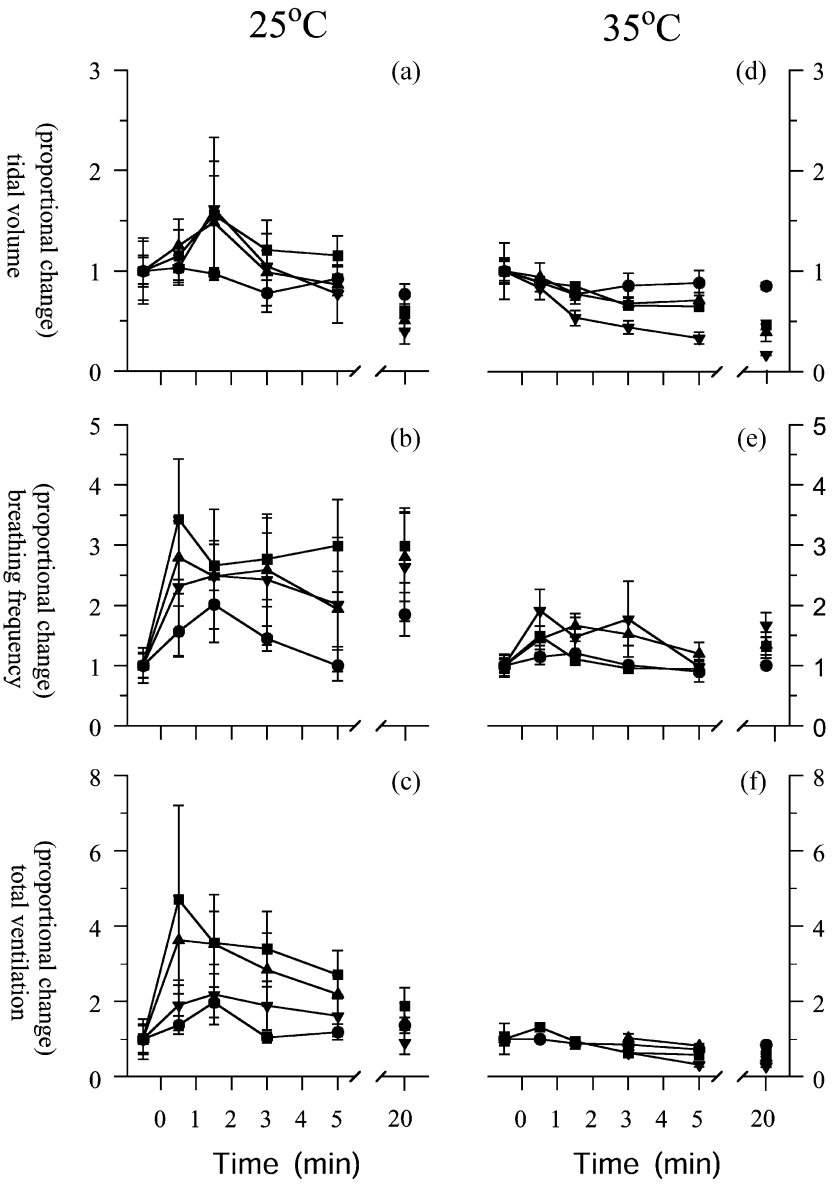


Fig. 5. The temporal development of the post-hypercapnic hyperpnea in *Uromastyx aegyptius* at 25 (●) and 35 °C (■). Values are expressed as proportional changes from the mean steady-state values recorded during exposure to hypercapnia (assumed to be 1). Inhalation of air following the hypercapnic exposure commenced at 0 min (inspired $CO_2$ 1%=●; 3%=■; 5%=▲; and 7%=▼). Values are presented as means ± 1 S.E.M. ($N=5$).

When the gular breaths were analysed separately, it seems that $CO_2$ stimulates their frequency, which is in contrast to the frequency of the aspiration pump. Thus, whilst filling of the lungs by aspiration seems sensitive to lung stretch receptors, inhibited by $CO_2$, this does not seem to be the case for the buccal pump, suggesting that *Uromastyx* does not rely on $CO_2$ sensitive pulmonary stretch receptors to regulate buccal breathing. This agrees with Al Ghamdi et al. (2001), who found that sudden lung inflation, which arrested buccal pumping, was not due to washout of $CO_2$ (and excitation of stretch receptors) since inflation with $CO_2$ rich gas produced the same effects.

*Uromastyx* exhibited a decrease in $f_R$ during hypercapnia at both temperatures. Thus, hypercapnia causes *Uromastyx* to breathe in a pattern that is characterised by large deep breaths interspersed

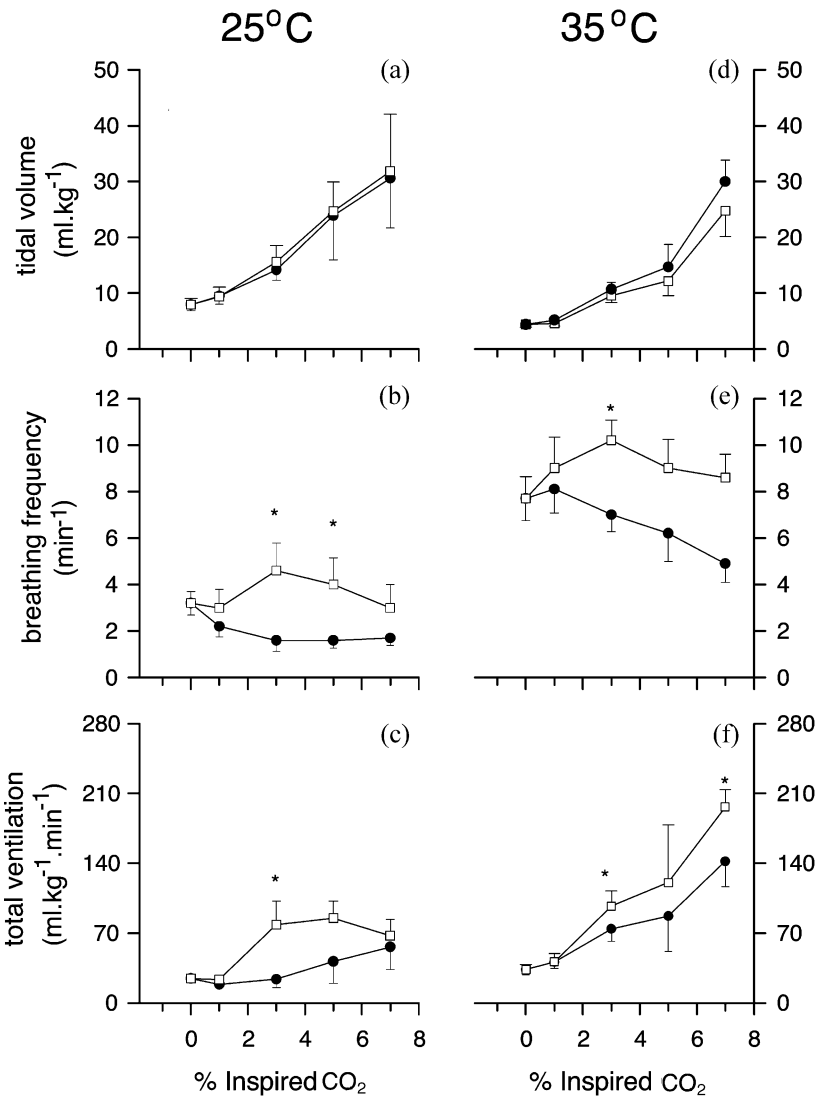


Fig. 6. The effect of the preceding $CO_2$ level in the inspired air on the immediate post-hypercapnic hyperpnea (1 min after switch back to air; open squares) as compared with steady state values recorded during exposure to hypercapnia (filled circles) in *Uromastyx aegyptius* at 25 and 35 °C. An asterisk indicates significant differences between steady state and the immediate post-hypercapnic hyperpnea values. Values are presented as means ± 1 S.E.M. ($N=5$).

by longer time intervals. Several earlier studies on reptiles have found similar reductions in $f_R$ (Nielsen, 1961; Glass and Johansen, 1976; Milsom, 1995a,b), while some other studies reported an increase particularly at lower levels of $CO_2$ (Milsom, 1995a,b). We did not observe any noticeable signs on the behaviour of the lizards during exposure to the high levels of hypercapnia, but it is possible that the large reductions in $f_R$ at 5 and 7% $CO_2$ may have been caused by a direct narcotic effect of $CO_2$ and/or the accompanying acidosis (e.g. Wang et al., 1993). Nevertheless, the reduction of $f_R$ at low levels seems to be caused by inhibitory $CO_2$ sensitive receptors (Milsom, 1995a,b). In most reptiles, inhibitory $CO_2$ receptors are located within the lungs (intrapulmonary chemoreceptors, IPC), which possess a discharge that is inversely related to $CO_2$ concentration and are insensitive to stretch of the lungs. In addition, snakes and lizards possess $CO_2$ sensitive chemoreceptors in the upper airways that also inhibit $f_R$ during hypercapnia (Ballam, 1985; Coates and

Ballam, 1987; Ballam and Coates, 1989; Coates and Ballam, 1989). The effects of temperature on upper airway receptors have not been addressed, but it has been shown that the sensitivity of IPCs in snakes, lizards and alligators increases with elevated temperature (Douse and Mitchell, 1988; Furilla and Bartlett, 1988; Douse et al., 1989). In addition, the neural activity arising from IPC stimulation increases more than that arising from PSR stimulation in *Thamnophis* and *Alligator* (Furilla and Bartlett, 1988; Douse et al., 1989). In *Uromastyx* the proportional changes in $f_R$ between room air and 7% $CO_2$ were the same at the two temperatures.

In reptiles, it has been reported that total ventilation may increase or decrease during hypercapnia (see Milsom, 1995a,b for reviews). These apparently opposing effects depend primarily on the extent to which $f_R$ is depressed or stimulated by hypercapnia. These changes, in turn, depend on whether the inhibitory input from IPC and upper airway receptors override the stimulatory input from vascular and central chemoreceptors that monitor pH and $P_{CO_2}$ of the blood. *Uromastyx* increased $\dot{V}_e$ during hypercapnia and this increase was more apparent at high temperature. This may suggest that the stimulatory effects arising from central and vascular chemoreceptors increase more with elevated temperature than the inhibitory input from IPCs and upper airway receptors. Thus, while experiments on *Alligator*, *Tupinambis* and *Thamnophis* show that IPC sensitivity increases with elevated temperature, it is also evident that the contribution from central chemoreceptor stimulation increases with elevated temperature (e.g. Branco and Wood, 1993).

### *4.2. Post-hypercapnic hyperpnea*

Ventilation increased immediately following the removal of $CO_2$ from the inspired air and this post-hypercapnic hyperpnea is similar to other species of reptiles, where $f_R$ generally increases and $V_T$ decreases progressively. This was certainly the case for *Uromastyx*. The post-hypercapnic hyperpnea follows the immediate removal of $CO_2$ from the lungs and upper airways, which abolishes the inhibitory input from the IPC and upper airway receptors. However, because the changes in pH and $CO_2$ of the blood follow a slower time course, the ventilatory drive through stimulation of vascular $CO_2$ receptors (central and peripheral) remains high during the initial recovery period. Thus, it is generally believed that the post-hypercapnic hyperpnea represents the excitatory input from these receptors in the absence of inhibitory input from chemoreceptors in the upper airways and lungs (Glass and Johansen, 1976; Milsom, 1995a,b). The relative contribution of IPC and upper airway receptors has not been determined. However, the bullfrog *Rana catesbeiana* does not appear to possess IPCs, and yet it exhibits a post-hypercapnic hyperpnea (Kinkead and Milsom, 1996). Thus a post-hypercapnic hyperpnea appears to have evolved early amongst air-breathing vertebrates and indeed has recently been demonstrated in the South American lungfish *Lepidosiren* (Sanchez and Glass, 2001).

In *Uromastyx*, the post-hypercapnic hyperpnea was present at both experimental temperatures. When expressed relative to the steady-state value obtained during hypercapnia, the post-hypercapnic hyperpnea was apparent at 25 °C but negligible at 35 °C (Fig. 5). In a study on *Coluber*, Nolan and Frankel (1982) also noted that the post-hypercapnic hyperpnea was large at 15 °C, but much less distinct at 25 °C and, in some cases, entirely absent at 35 °C. These observations may seem surprising because the sensitivity of IPC increases with temperature (Douse and Mitchell, 1988; Furilla and Bartlett, 1988; Douse et al., 1989). However, the relative change in IPC activity to a given stimulus was not affected by temperature in *Thamnophis* and *Tupinambis* (Douse and Mitchell, 1988; Furilla and Bartlett, 1988). It is possible that the low post-hypercapnic hyperpnea at high temperature reflects a faster time course of $CO_2$ washout from blood, lungs and upper airways, due to increased pulmonary perfusion and higher steady state levels of ventilation.

### *4.3. Evidence for a contribution of buccal pumping to pulmonary ventilation*

A recent study on lightly anaesthetised *Uromastyx* (Al Ghamdi et al., 2001), demonstrated that contraction of the buccal cavity can contribute significantly to inflation of the lungs. This mechanism resembles the breathing mechanism of amphibians, and is in addition to the thoracic aspiratory pump that normally characterises ventilatory mechanics in reptiles. There are several early observations of buccal pumping causing lung inflation in lizards, though this was often consid-

ered to have a protective rather than a respiratory role (e.g. Salt, 1943; Templeton, 1967). The use of buccal or gular pumping in *Uromastyx* appeared to be recruited at high temperatures when the ventilatory efforts were elevated, presumably in response to increased oxygen demand (Al Ghamdi et al., 2001). Similarly, varanid lizards employ buccal breathing during and after exercise (Owerkowicz et al., 1999), which may contribute to overcome the supposed mechanical constraints on the use of hypaxial muscles for ventilation (Carrier, 1987; Owerkowicz et al., 1999). In the present study, *Uromastyx* often exhibited a breathing pattern that was characterised by a single costal breath followed by a number of consecutive smaller inspiratory excursions (Fig. 3). This breathing pattern closely resembles the airflows recorded during buccal breathing by Al Ghamdi et al. (2001), suggesting that this response also occurs in fully recovered and conscious animals. We found that this response was conspicuous at high levels of hypercapnia and particularly so during the post-hypercapnic hyperpnea. This observation agrees with the notion that buccal breathing is recruited to supplement $V_{T,}$ whenever high levels of ventilation are required.

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