Thermoregulatory role of insensible evaporative water loss constancy in a heterothermic

marsupial

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Abstract

"Insensible" evaporative water loss of mammals has been traditionally viewed as a passive process, but recent studies suggest that insensible water loss is under regulatory control, although the physiological role of this control is unclear. We test the hypothesis that regulation of insensible water loss has a thermoregulatory function by quantifying for the first time evaporative water loss control, along with metabolic rate and body temperature, of a heterothermic mammal during normothermia and torpor. Evaporative water loss was independent of ambient relative humidity at ambient temperatures of 20 and 30°C, but not at 25°C or during torpor at 20°C. Evaporative water loss per water vapour pressure deficit had a positive linear relationship with relative humidity at ambient temperatures of 20 and 30°C, but not at 25°C or during torpor at 20 or 25°C. These findings suggest that insensible water loss deviates from a physical model only during thermoregulation, providing support for the hypothesis that regulation of evaporative water loss has a thermoregulatory role.

Introduction

"Insensible" water loss is evaporative water loss (EWL) that results from the inevitable permeability of skin and lung epithelia to water vapour [1] at ambient temperatures (T_a) in and below the thermoneutral zone (TNZ). It has traditionally been viewed as a passive process, determined physically by the water vapour pressure deficit (ΔWVP) between the animal and the ambient air [2,3,4]. However, the ability to maintain insensible EWL independent of environmental conditions that modify the ΔWVP has recently been recognised for mammals [5,6].

Changing the ambient relative humidity (RH) at a particular T_a perturbs the evaporative environment of an animal; the physical model predicts a linear decrease in EWL with increasing RH (or WVP, as RH and WVP are equivalent measures of "humidity" at a constant T_a). However deviations from this model are difficult to test as the expected slope for EWL as a function of RH is unknown. Expressing EWL as a function of ΔWVP resolves this; EWL/ΔWVP should be independent of RH, with a slope of 0.

However, there is a computational complication regarding the temperature used to calculate the animal's saturation WVP, hence ΔWVP and EWL/ΔWVP. The appropriate temperature is a combination of skin and expired air temperature, weighted by the relative partitioning of respiratory and cutaneous EWL, but these are difficult to measure accurately [7]. Therefore ΔWVP calculated from WVP saturation at both body temperature (T_b) and T_a, minus ambient WVP, provide lower and upper limits respectively, with EWL/ΔWVPT_b being a conservative but probably more realistic estimate, such that the slope of it vs RH ≠ 0 is strong evidence of EWL control [6].

The specific physiological role of insensible EWL control is unknown. One hypothesis is that maintaining EWL constant over a range of ΔWVP simplifies thermoregulation [5,6]. Changes in EWL in response to a changing ΔWVP will modify evaporative heat loss (EHL), requiring a regulatory adjustment of heat production if T_b is to remain constant. However, if EWL is maintained constant under different evaporative conditions, then T_b and/or metabolic heat production (MHP) will not be impacted. Here we test this hypothesis by examining for the first time constancy of EWL at varying RH during both normothermia and torpor for a heterothermic dasyurid marsupial, the red-tailed phascogale (*Phascogale calura*), separately at three ambient temperatures.

Methods

Ten red-tailed phascogales were captured at Dryandra (31° 46' S, 117° 1' E), Western Australia. Metabolic rate (MR; oxygen consumption, VO_2 and carbon dioxide production, VCO_2) and EWL were measured by flow-through respirometry at T_as of 20, 25 and 30°C (at and below thermoneutrality [8]) and RHs of approximately 17, 36, 48, 63 and 81%, following [6], exploring as close to the entire range of dry to saturated conditions at each T_a as logistically possible. Six to 10 post-absorptive phascogales were measured at each RH and T_a combination during their rest phase.

The VO_2, VCO_2 and EWL were calculated after [9]. Phascogales were considered torpid if MR was <70% of normothermic MR. The ΔWVP between the animal and ambient air was calculated (using hygrometric equations [10]) as the difference between the ambient WVP at the measured RH and T_a and saturation WVP (the animal), using either T_b (ΔWVPT$_b$) or T_a (ΔWVPT$_a$) as the animal's evaporative temperature. For normothermic phascogales, T_b was measured at the conclusion of experiments using a thermocouple inserted into the cloaca. Wet (C_{wet}) and dry (C_{dry}) thermal conductance were calculated as MHP/(T_b-T_a) and (MHP-EHL)/(T_b-T_a) respectively, where MHP is metabolic heat production calculated from O_2 converted to J using the oxycalorific coefficient for the measured RER (VCO_2/VO_2) e.g. interpolated from 19.5 J mL O_2^{-1} at RER = 0.7 and 20.1 J mL O_2^{-1} at RER = 1. Torpor T_b was calculated as $T_b = T_a + ((MHP-EHL)/C_{dry})$, to enable calculation of ΔWVPT$_b$.

Values are mean ± SE, with N = individuals and n = measurements. Impacts of RH on torpor frequency were assessed with a log-likelihood goodness-of-fit test (statistiXL V2). Linear mixed-effect models were used to examine RH effects on physiological variables while accounting for individual. We used the lmer function in lme4 [11], along with lmerTest [12] to determine probabilities, in R Studio [13] to assess the significance of RH as a fixed factor, and individual as a random factor, at each T_a. The lme function in nlme [14] was used to assess the significance of reverse Helmert *a priori* contrasts, with individual as a random factor.

Results

The mean mass of phascogales over all experiments was 32.3 ± 0.52g (N = 10, n = 99). Phascogales remained normothermic at T_a = 30°C, but entered torpor during 16 (of 32) measurements at T_a = 25°C and 20 (of 38) at T_a = 20°C. There was no significant effect of RH on torpor frequency ($G_4 \leq 5.52$, $p \geq 0.238$). There were no significant individual differences for any physiological variables ($\chi_1^2 \leq 2.6$, $p \geq 0.10$) except T_b at T_a = 30°C ($\chi_1^2 = 5.62$, $p = 0.02$).

For normothermic phascogales, EWL was independent of RH at T_a = 20 and 30°C ($F_{1,25-29} \leq 2.13$, $p \geq$ 0.123), but not at T_a = 25°C ($F_{1,32}$ = 23.2, p < 0.001). *A priori* contrasts indicated that at T_a = 20°C EWL was lower at the highest RH (t_{22} = 7.05, P < 0.001). EWL of torpid phascogales was dependent on RH at T_a = 25 ($F_{1,16}$ < 15.5, p = 0.001) but not T_a = 20°C ($F_{1,20}$ = 3.55, p = 0.074).

The EWL/$\Delta WVPT_b$ of normothermic phascogales had a positive linear relationship at T_a = 20 (Figure 1; $F_{1,24}$ = 6.95, p = 0.014) and 30°C ($F_{1,34}$ = 11.3, p = 0.002) but not T_a = 25°C ($F_{1,32}$ = 0.576, p = 0.453). These relationships were described by the equations EWL/$\Delta WVPT_b$ = 0.25RH + 40.2 at 20°C and EWL/$\Delta WVPT_b$ = 0.49RH + 30.6 at 30°C. In contrast, all T_a models for EWL/$\Delta WVPT_a$ vs RH were significant ($F_{1,24-32} \geq 18.4$, p $\leq$ 0.001). During torpor there were no significant linear relationships for EWL/$\Delta WVPT_b$ ($F_{1,16-20} \leq 0.435$, $p \geq 0.519$) or EWL/$\Delta WVPT_a$ ($F_{1,16} \leq 1.16$, $p \geq 0.253$) with RH at T_a = 20 or 25°C.

For normothermic phascogales, there were no RH effects (Figure 2) on VO_2 ($F_{1,24-28} \leq 4.14$, $p \geq 0.051$) or T_b ($F_{1,25-32} \leq 2.84$, $p \geq 0.103$) at any of the T_a, although at T_a = 30°C the VO_2 at RH = 81% was higher than at other RH (*a priori* contrast, t_{21} = 3.19, p = 0.004). Neither C_{wet} ($F_{1,25-28} \leq 0.1.84$, $p \geq 0.187$) nor C_{dry} ($F_{1,25-28} \leq 2.03$, $p \geq 0.166$) were influenced by RH at any T_a, but *a priori* contrasts indicated that both C_{wet} and C_{dry} were higher at RH = 81% compared to lower RH ($t_{22} \geq 2.72$, p $\leq$ 0.013). During torpor, VO_2 was independent of RH at both T_a = 20 and 25°C ($F_{1,16} \leq 0.3.25$, $p \geq 0.090$).

Discussion

Our data provide clear evidence that the insensible EWL of red-tailed phascogales does not necessarily follow a purely physical model (i.e. constant EWL/ΔWVP). At T_a = 20 and 30°C there were unequivocal deviations from expected passive responses to changes in RH. Patterns of EWL control with T_a during normothermia suggest that this occurs, or is most pronounced, at T_a where changes in EHL may impact on thermoregulation. However, EWL control was equivocal at T_a = 25°C, close to but below the lower critical temperature (T_{lc}). During torpor, when phascogales were thermoconforming, we found no

clear evidence of EWL control. These patterns support the hypothesis that EWL regulation has a thermoregulatory role that maintains EHL constant under conditions that may perturb the evaporative environment.

At $T_a > T_{lc}$, EWL is critical to adjustments in heat balance that maintain the TNZ. As MHP cannot be reduced in thermoneutrality, inhibition of EHL at low ΔWVP to the same EWL/ΔWVPT$_b$ as at high ΔWVP (i.e. the physical model) would increase T_b. Therefore, to maintain T_b constant, EWL remained constant at a range of ΔWVP, so EWL/ΔWVPT$_b$ must vary. At T_a = 25°C, where we couldn't unequivocally demonstrate EWL control, phascogales were close to T_{lc}. Inhibition of EHL at lower ΔWVP would have the effect of shifting their thermal balance closer to thermoneutrality. However we calculate this shift to be small, equivalent to an increase in T_a of only 0.4°C for the lowest ΔWVP, based on the relationship between VO_2 and T_a [8].

For normothermic phascogales at T_a below thermoneutrality (e.g. 20°C), increased ventilatory minute volume (to accommodate increased MR) should increase insensible EWL above that at T_a = 30°C in the absence of EWL regulatory control. If respiratory EWL was 50% of total EWL and ventilation increased 4 fold [8] (and cutaneous EWL remained constant), then EWL/ΔWVPT$_b$ and EHL would increase 2.5 fold. However, EWL is constant at a range of ΔWVP at low T_a, and this regulation maintains EHL constant despite increased ventilation. Similarly, the reduction of EWL/ΔWVP at low RH and low T_a allows phascogales to maintain a constant EHL, hence stable T_b, without adjustments to heat production (VO_2).

The occurrence of torpor was not impacted by RH, indicating that phascogales do not avoid torpor in less favourable evaporative environments. Our failure to find unequivocal evidence of EWL control for thermoconforming torpid phascogales adds further support to the hypothesis that control of insensible EWL has a thermoregulatory function, occurring only during active thermoregulation.

Conclusion

We present evidence of a thermoregulatory role for control of insensible EWL by a small heterothermic marsupial. Consistent patterns in control of insensible EWL related to thermoregulatory requirements indicate that thermoregulating phascogales maintain a constant insensible EWL, avoiding the potential impacts on heat loss of physically determined EWL. This simplifies thermoregulation under otherwise perturbing ambient conditions of changing RH and ΔWVP.

Ethics

Experiments followed the Australian Code of Practise for the care and use of animals for scientific purposes, approved by Curtin animal ethics committee (AEC_2016_01). Phascogales were caught and held under licence from Department of Parks and Wildlife (SF010573, CE005075).

Data, code and materials

Datasets supporting this article have been uploaded as supplementary material.

Competing Interests

We have no competing interests

Authors' contributions

Both authors made substantial contributions to the study conception and design, data acquisition, analysis and interpretation, and drafting and revising the article. Both gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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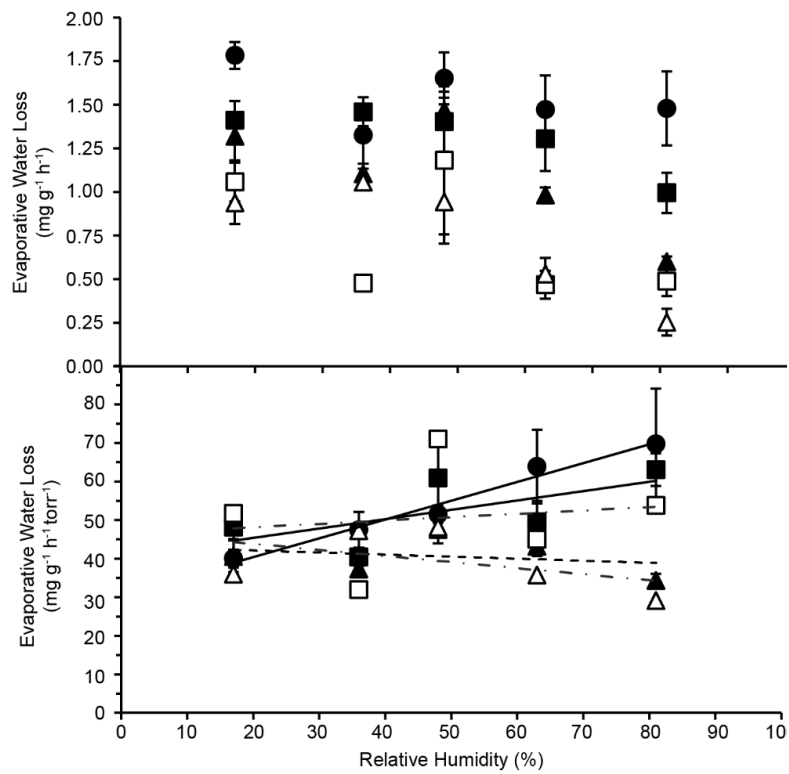
Figure One

Evaporative water loss (top panel) of red-tailed phascogales (*Phascogale calura*) during normothermia (black symbols) and torpor (white symbols) at ambient temperatures of 20 (squares), 25 (triangles) and 30°C (circles) at various ambient relative humidities and (bottom panel) as a function of the water vapour pressure differential at different relative humidities. Solid lines indicate significant linear relationships, dashed lines non-significant i.e. slope = 0 relationships for normothermic (black) and torpid (grey) phascogales. Values are mean ± SE, N = 10, n = 6-7.
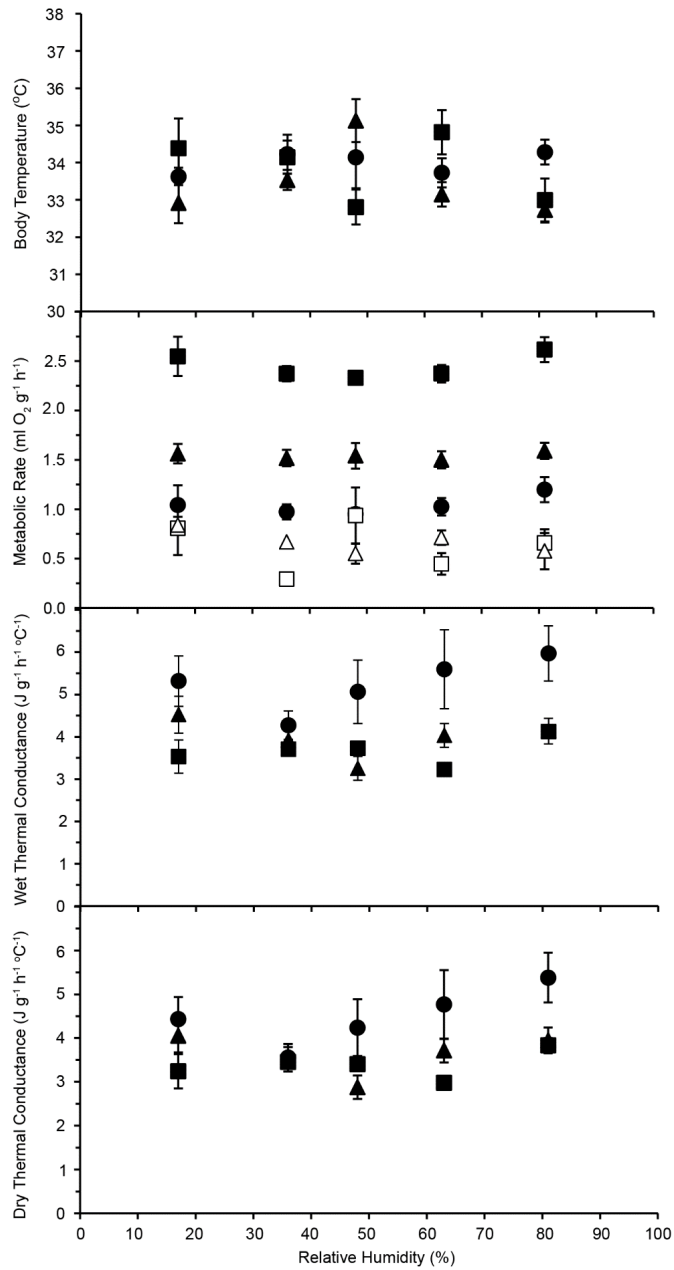
Figure Two

Body temperature, metabolic rate and wet and dry thermal conductance of normothermic (black symbols) and torpid (white symbols) red-tailed phascogales (*Phascogale calura*) at ambient temperatures of 20 (squares), 25 (triangles) and 30°C (circles) and various ambient relative humidities. Values are mean ± SE, N = 10, n = 6-7.