Physiological responses of a rodent to heliox reveal constancy of evaporative water loss under perturbing environmental conditions

Christine Elizabeth Cooper and Philip Carew Withers

Department of Environment and Agriculture, Curtin University Perth, Western Australia; and Animal Biology, University of Western Australia, Crawley, Western Australia

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Cooper CE, Withers PC. Physiological responses of a rodent to heliox reveal constancy of evaporative water loss under perturbing environmental conditions. Am J Physiol Regul Integr Comp Physiol 307: R1042–R1048, 2014. First published August 27, 2014; doi:10.1152/ajpregu.00051.2014.—Total evaporative water loss of endotherms is assumed to be determined essentially by biophysics, at least at temperatures below thermoneutrality, with evaporative water loss determined by the water vapor deficit between the animal and the ambient air. We present here evidence, based on the first measurements of evaporative water loss for a small mammal in heliox, that mammals may have a previously unappreciated ability to maintain acute constancy of total evaporative water loss under perturbing environmental conditions. Thermoregulatory responses of ash-grey mice (Pseudomys albocinereus) to heliox were as expected, with changes in metabolic rate, conductance, and respiratory ventilation consistent with maintaining constancy of body temperature under conditions of enhanced heat loss. However, evaporative water loss did not increase in heliox. This is despite our confirmation of the physical effect that heliox augments evaporation from nonliving surfaces, which should increase cutaneous water loss, and increases minute volume of live ash-grey mice in heliox to accommodate their elevated metabolic rate, which should increase respiratory water loss. Therefore, mice had not only a thermoregulatory but also a hygroregulatory response to heliox. We interpret these results as evidence that ash-grey mice can acutely control their evaporative water loss under perturbing environmental conditions and suggest that hygroregulation at and below thermoneutrality is an important aspect of the physiology of at least some small mammals.

ash grey mouse; endotherm; evaporative water loss; heliox; metabolic rate; regulation; ventilation

MAINTENANCE OF WATER BALANCE is a core physiological process for animals, so hygic physiology is under substantial selection pressure and is of particular adaptive significance for terrestrial species. Evaporative water loss (EWL) is an important component of a terrestrial endotherm’s overall water budget; it can be 70% or more of the total water loss (13, 25, 48). Consequently, it is important to understand the determinants of EWL in animals. Total EWL includes water evaporated across the body surface (cutaneous EWL) and the respiratory surface (respiratory EWL). Although the relative proportions of these avenues vary with biological and environmental factors (54), it has been a central paradigm of animal physiology that total EWL for mammals and birds is determined essentially by physical processes, at least at ambient temperatures (T_a) below the lower critical temperature of the thermoneutral zone (T_{tc}). It has been assumed that the water vapor pressure deficit (Δ wvp) between the animal and the ambient air is a primary determinant of EWL (9). A small Δ wvp should retard EWL, and a large Δ wvp should enhance EWL (22, 34). This expected inverse and linear relationship has been demonstrated previously for a variety of mammals and birds (2, 9, 15, 22, 47). Consequently, there is no known mechanism for an endotherm to regulate a constant evaporative water loss at T_a below thermoneutrality, under perturbing environmental conditions, such as varying Δ wvp (in contrast to the well-accepted Scholander-Irving model of a negative feedback control system for thermoregulation) (27). However, at T_a above the T_{tc}, EWL is typically modulated for negative feedback thermoregulatory control (e.g., panting, sweating, and salivation) (29).

Some studies of small mammals and birds (4, 12, 34, 53) have reported rates of EWL that were unexpectedly independent of ambient Δ wvp, at least at low T_a. These observations, coupled with the finding that correcting EWL measurements of marsupials for environmental Δ wvp did not improve the allometric scaling of EWL (it actually made the allometric relationship more variable) (12) suggest that some endotherms may be able to maintain constancy of EWL under perturbing environmental conditions.

Previous observations of EWL constancy were made with relative humidity (RH) and T_a manipulated to vary the Δ wvp between the animal and its environment, thereby changing the physical gradient driving evaporation. Here, we use a heliox mixture (21% oxygen in helium) to perturb the evaporative environment of a small mammal, the ash-grey mouse (Pseudomys albocinereus), to further investigate the potential for regulation of EWL independent of RH and T_a.

Heliox is commonly used to increase heat loss from animals, as helium has a thermal conductivity 6.5 times that of nitrogen. Heliox is 4 times more conductive than air and increases heat flux between the animal and its environment (19, 23, 37, 42). For thermoregulating endotherms, this increase in heat loss requires a proportional increase in metabolic heat production (MHP) (19, 37, 42), but it is generally assumed that heliox does not alter cellular metabolic processes (19, 30, 33) and has no effect on an animal’s evaporative heat loss (EHL) (19, 23, 37). Consequently, observed metabolic effects of heliox on endothermic animals are attributed solely to a thermoregulatory response to increased heat loss. However, water diffuses 2.3 times faster in heliox compared with air, due to the density of helium being 1/7 that of nitrogen at standard temperature and pressure (21, 30, 32, 33). Therefore, we expect EWL of a small mammal below thermoneutrality (if it is determined purely by biophysical mechanisms) to be higher in heliox than in air, due to both enhanced diffusion and increased respiratory ventilation. Helium mixtures have been used previously to modify the evaporative environment of plants (16,
MAINTAINING CONSTANCY OF EVAPORATIVE WATER LOSS

Eight adult male ash-grey mice (*P. albocinctus*) were captured in pit traps at Bungalbin, Western Australia (30° 20’S, 119° 45’E) during late December 2011. Mice were housed indoors at Curtin University, with a 12:12-h light-dark cycle, at a *T*<sub>a</sub> of ~22°C. They were fed seed, mouse cubes, and fresh fruit and vegetables, and provided with ad libitum water. All experiments were performed according to the Australian Code of Practise for the Care and Use of Animals for Scientific Purposes. Mice were captured and held under license from the Western Australian Department of Parks and Wildlife and the study was approved by the Curtin University Animal Ethics Committee.

Mice were fasted for at least 12 h before the commencement of experiments. Open flow-through respirometry was used to measure metabolic rate (V<sub>O2</sub>, V<sub>CO2</sub>) and EWL of the ash-grey mice during their inactive phase (day), until V<sub>O2</sub>, V<sub>CO2</sub>, and EWL were stable and minimal. At the end of the experiment, body temperature (*T*<sub>a</sub>) was measured using a plastic-tipped type-K thermocouple (connected to a Vaisala temperature sensor and platinum resistance *Ta* probe (Vaisala HMP45A; Helsinki, Finland); RH range 0 – 100%, accuracy ± 1% RH). EWL was measured for four dead mice was higher in heliox than air. V<sub>O2</sub> measured in air at *Ta* varied by 10.2 ± 0.33% on July 5, 2017 http://ajpregu.physiology.org/ Downloaded from AJP-Regul Integr Comp Physiol • doi:10.1152/ajpregu.00051.2014 • www.ajpregu.org

RESULTS

Mean body mass of mice measured for all experiments (*N* = 8, *n* = 64) was 28.2 g (SD 3.6). Body temperature was then warming it to ambient temperature and comparing the theoretical and measured RH (12). This calibration was then routinely confirmed using 1% RH air (dried with Drierite) and 100% RH air (saturated; by breathing on the probe). The Vaisala temperature sensors and thermocouple meter were calibrated against a thermometer with calibration traceable to a national standard. Flow meters were volumetrically calibrated using a Sensidyne Glibilibrator 2 (Clearwater, FL), separately for both air and heliox. The plethysmograph system was calibrated by injecting a known volume (0.05 ml) of air into the chamber after the last ventilatory measurement and determining the pressure displacement and washout characteristics of the resulting pulse (41).

A custom-written VB program was used for metabolic and hygienic calculations (51). V<sub>O2</sub>, V<sub>CO2</sub>, and EWL were calculated for each mouse for a 20-min period when all three variables were constant and minimal, using the equations: V<sub>O2</sub> = V<sub>r</sub>(F<sub>IO2</sub> − [F<sub>ECO2</sub> − (1 − F<sub>IO2</sub> − F<sub>CO2</sub>)]) / (1 − F<sub>IO2</sub> − F<sub>CO2</sub>), V<sub>CO2</sub> = V<sub>r</sub>([F<sub>ECO2</sub> − (1 − F<sub>IO2</sub> − F<sub>CO2</sub>)] + F<sub>ECO2</sub>), and EWL = V<sub>r</sub>[F<sub>H2O</sub> / (1 − F<sub>IO2</sub> − F<sub>ECO2</sub> − F<sub>H2O</sub>)] / (1 − F<sub>ECO2</sub> − F<sub>H2O</sub> − F<sub>ECO2</sub>)<sup>2</sup>, where *t* denotes current, *e* denotes excurrent, *V* denotes flow rate, and *F* denotes the fractional composition of air for that gas. Respiratory exchange ratio (RER) was calculated as V<sub>CO2</sub>/V<sub>O2</sub>, C<sub>water</sub> = (J·h<sup>−1</sup>·°C<sup>−1</sup>) was calculated as MHP/(T<sub>a</sub> − *T*<sub>e</sub>), with MR converted to MHP using the measured RER (interpolated from Table 4.2 of Ref. 50). C<sub>water</sub> = (MHP − EHL)/(T<sub>a</sub> − *T*<sub>e</sub>), with EHL calculated from EWL assuming 2.4 J/mg H<sub>2</sub>O (28, 50). Calculations for *f*<sub>ref</sub>, *V<sub>r</sub>*, minute volume (*V<sub>r</sub>*), and oxygen extraction (E<sub>O2</sub>) were made using a custom written VB (v6) data analysis program.

Values are presented as means (SD), with *N* = number of individuals and *n* = number of measurements. Both *N* and *n* = 8 unless stated otherwise. Gas volumes are presented at standard temperature and pressure dry, except *V<sub>r</sub>* and *V<sub>i</sub>* are at body temperature and pressure saturated. Effects of ambient gas composition (air or heliox) and *T*<sub>a</sub> (from 15–30°C) on physiological variables were examined using a full-factorial two-factor multivariate repeated-measures analysis of variance (RMANOVA) and a priori polynomial contrasts (52). Comparison of thermonuclear V<sub>O2</sub> and V<sub>CO2</sub> in air and heliox was made with a two-tailed paired *t*-test (if necessary correcting for equality of variance). The relationship between EWL from a plastic vial in air and heliox at a range of *Ta* was examined with analysis of variance (ANCOVA), while a one-tailed *t*-test to confirm that data at 30°C were within the thermoneutral zone of ash-grey mice (2) (see Fig. 1). Data at *T*<sub>a</sub> = 32.5°C were not included in further analyses or calculations as 32.5°C was clearly well above the *T*<sub>a</sub> of the thermoneutral zone (Fig. 1). Statistical analyses were conducted with SPSS (v11.0 for Windows) and statistiXL (v 1.10).

We used a sensitivity analysis to determine the possible range of expected (biophysical) heliox effects on total EWL for different combinations of respiratory and cutaneous partitioning. We assumed that partitioning would be somewhere between 80% cutaneous and 20% respiratory to vice versa (7, 8, 13, 15, 36, 43, 44, 54, 56), so we calculated predicted heliox effects for 20, 40, 60, and 80% cutaneous EWL (with the remainder respiratory). We modeled the heliox effect on cutaneous EWL from no effect (1 times increase in heliox compared with air) to a completely diffusional effect (2.3 times increase in heliox compared with air). Respiratory EWL would increase in proportion to *V<sub>i</sub>* in heliox compared with air (in this case 1.6 times), and we modeled the direct heliox effect ranging from 1 times to a maximum of 2.3 times.
independent of both ambient gas composition \(F_{1,7} = 1.45; P = 0.268\) and \(T_a (F_{3,5} = 1.76; P = 0.328)\), with a mean \(T_a\) for all mice in both air and heliox and at all \(T_a (N = 8, n = 64)\) of 34.9°C (SD 0.60; Fig. 1A).

Ambient gas composition had a significant influence on both wet and dry thermal conductance \((C_{\text{wet}}, F_{1,7} = 204; P < 0.001; C_{\text{dry}}, F_{1,7} = 180; P < 0.001)\), with both being 1.6 times higher in heliox compared with air (Fig. 1, B and C) at \(T_a\) below thermoneutrality. The overall \(T_a\) effect was significant for \(C_{\text{wet}} (F_{3,5} = 26.4; P = 0.01)\), which ranged from 113 J·h\(^{-1}\)·°C\(^{-1}\) (SD 25.3) at \(T_a = 15°C\) to 187 J·h\(^{-1}\)·°C\(^{-1}\) (SD 63.9) at \(T_a = 30°C\) in air, but not \(C_{\text{dry}} (F_{3,5} = 9.66; P = 0.143)\), which ranged from 108 J·h\(^{-1}\)·°C\(^{-1}\) (SD 23.9) to 164 J·h\(^{-1}\)·°C\(^{-1}\) (SD 20.2). There was a significant positive linear contrast for \(C_{\text{wet}}\) with \(T_a (F_{1,7} = 29.2; P = 0.001)\) and a significant quadratic contrast for \(C_{\text{dry}} (F_{1,7} = 16.4; P = 0.005)\).

Oxygen consumption \((\dot{V}O_2)\) increased in air from 1.51 ml O\(_2\)·g\(^{-1}\)·h\(^{-1}\) (SD 0.37) at \(T_a = 30°C\) to 4.02 (SD 0.20) at \(T_a = 15°C\), and in heliox from 1.55 ml O\(_2\)·g\(^{-1}\)·h\(^{-1}\) (SD 0.34) at \(T_a = 30°C\) to 6.61 ml O\(_2\)·g\(^{-1}\)·h\(^{-1}\) (SD 2.44) at \(T_a = 15°C\) (Fig. 1D). There were highly significant effects on \(\dot{V}O_2\) of both ambient gas composition and \(T_a (F_{3,5} = 101; P < 0.001)\) and \(T_a (F_{3,5} = 72.7; P < 0.001)\), and there was also a significant interaction between ambient gas composition and \(T_a (F_{3,5} = 42.2; P = 0.001)\). \(T_a\) effects were described by a significant negative linear contrast \((F_{1,7} = 241; P < 0.001)\). There was no significant difference between \(\dot{V}O_2\) at \(T_a = 30\) and 32.5°C \((t = 0.210; P = 0.840)\), confirming that 30°C was within the thermoneutral zone (see also Ref. 2). At a thermoneutral \(T_a\) of 30°C, there was no significant difference between \(\dot{V}O_2\) measured in air or heliox \((t = 0.210; P = 0.840)\), but \(\dot{V}O_2\) in heliox was 1.6 times that in air at lower \(T_a\). Patterns of \(\dot{V}O_2\) were similar to those of \(\dot{V}O_2\), with significant effects for ambient gas composition \((F_{1,56} = 31.7; P < 0.001)\) and \(T_a (F_{3,56} = 91.7; P < 0.001)\) and the interaction \((F_{3,56} = 6.39; P = 0.001)\;\text{Fig. 1E) Again, there was no significant difference between \(\dot{V}O_2\) measured at thermoneutrality in air or heliox \((t = 1.44; P = 0.201)\), but \(\dot{V}O_2\) in heliox was 1.4 times that in air at lower \(T_a\).

Both \(T_a\) and ambient gas composition affected respiratory ventilation (Fig. 2, A–D). \(f_R\) was influenced by ambient gas composition \((F_{1,7} = 81.2; P < 0.001)\) and \(T_a (F_{3,5} = 59.3; P = <0.001)\) with a significant interaction between these factors \((F_{3,5} = 8.64; P = 0.020)\). The \(T_a\) effect was described by a significant negative linear contrast \((F_{3,5} = 75.6; P < 0.001)\), with \(f_R\) ranging from 69.4 breaths/min (SD 12.5) at \(T_a = 30°C\) to 154 breaths/min (SD 38.5) at \(T_a = 15°C\) in air, and 85.3 breaths/min (SD 21.6) to 227 breaths/min (SD 26.3) over the same \(T_a\) range in heliox, \(f_R\) was 1.4 times higher in heliox than in air at \(T_a\) below thermoneutrality. Ambient gas composition also influenced tidal volume \((V_T; F_{1,7} = 12.8; P = 0.009)\), with \(V_T\) 1.2 times higher in heliox at the two lowest \(T_a [0.217 ml (SD 0.033) in air and 0.269 ml (SD 0.058)] in heliox at \(T_a = 15°C\), and 0.236 ml (SD 0.057) in air and 0.285 ml (SD 0.085) in heliox at \(T_a = 20°C\), but there was no \(T_a\) effect \((F_{3,5} = 0.627; P = 0.628)\). \(V_T\) was influenced by both ambient gas composition \((F_{1,7} = 82.3; P < 0.001)\) and \(T_a (F_{3,5} = 31.8; P = 0.001)\), and there was a significant interaction \((F_{3,5} = 12.8; P = 0.009)\). The \(T_a\) effect was described by a significant negative linear contrast \((F_{3,5} = 129; P < 0.001)\); \(V_T\) ranged from 17.5 ml/min (SD 3.05) at \(T_a = 15°C\) to 32.6 ml/min (SD 4.5) at \(T_a = 30°C\) in air, and from 21.8 ml/min (SD 3.76) to

![Fig. 1. Physiological responses of ash-grey mice (Pseudomys albocinereus) in air (o) and heliox (c). Values are expressed as means ± SE (for ease of comparing means); n = 8. Body temperature (Tb) was independent of both ambient temperature and gas (A), but there were significant effects of a heliox atmosphere on wet (Cwet; B) and dry (Cdry; C) thermal conductance and metabolic rate [oxygen consumption, VO2 (D) and carbon dioxide production VCO2 (E)].](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00051.2014)
respectively; Fig. 3), although there was a significant negative linear contrast for $T_a$ ($F_{3,5} = 9.78; P = 0.017$). EWL ranged from 1.52 mg·g$^{-1}$·h$^{-1}$ (SD 0.40) at $T_a = 30^\circ$C to 1.87 mg·g$^{-1}$·h$^{-1}$ (SD 0.53) at $T_a = 20^\circ$C in air, and from 1.41 mg·g$^{-1}$·h$^{-1}$ (SD 0.29) to 2.03 mg·g$^{-1}$·h$^{-1}$ (SD 0.73) over the same $T_a$ range in heliox. For a perforated, water-filled vial, there were significant linear relationships between EWL and $T_a$ in both heliox and air ($F_{1,6} = 14.9; P = 0.008; F_{1,12} = 47.3; P < 0.001$, respectively). These relationships had parallel slopes (slope test $F_{1,18} = 2.54, P = 0.128$), but the elevation of the relationship for EWL in heliox was significantly higher (1.7 times) than that for ambient air ($F_{1,19} = 73.1; P < 0.001$). For dead mice, EWL was 0.60 mg·g$^{-1}$·h$^{-1}$ (SD 0.18) in air and 1.75 mg·g$^{-1}$·h$^{-1}$ (SD 1.32) in heliox; this significant difference ($t_{3,1} = 2.43; P = 0.047$) reflects a 2.9 times higher EWL in heliox than air.

Our sensitivity analysis (Fig. 3) indicated that the effects of heliox at all $T_a$ would be greatest if there were a purely diffusional effect (2.3 times) of heliox on both cutaneous and respiratory EWL, and minimal if there were no direct heliox effect on cutaneous or respiratory EWL, just a 1.6 times increase in respiratory EWL due to the observed increase in $V_t$. Under the unlikely (not supported by our data for dead mice or the literature) situation that there is no enhanced diffusive component to cutaneous or respiratory EWL, EWL in heliox would range from as little as 1.1 times that in air if respiratory EWL were only 20% of total EWL, to 1.5 times that in air if respiratory EWL is 80% of total EWL. The heliox effect is much more exaggerated when a 2.3 times diffusive effect is applied to either the cutaneous component or both the respiratory and cutaneous components of EWL. The actual heliox effect should fall somewhere between these extremes.
DISCUSSION

We present here the first measurements of EWL for a small endotherm in heliox, demonstrating an ability to maintain acute constancy of total EWL under this perturbing environmental condition. Thermoregulatory responses of ash-grey mice to a heliox atmosphere were as expected, and changes in thermal conductance, MR, and ventilatory variables were all consistent with maintaining $T_b$ constant under conditions of enhanced (1.6 times) heat loss. However, mice demonstrated not only thermoregulatory constancy of $T_b$, but also constancy of EWL that we hypothesize may reflect a hygroregulatory response to a heliox environment.

Ash-grey mice regulated $T_b$ independent of ambient gas composition and $T_a$, remaining normothermic under all measurement conditions. Therefore, there are no potential complications in interpreting gas or $T_a$ effects that include heterothermic individuals. Measurements in air at $T_a = 32.5^\circ C$ confirmed that $T_a = 30^\circ C$ was within the thermoneutral zone for this species (1), so our comparisons of air and heliox are indeed (1.6 times) heat loss. However, mice demonstrated not only thermoregulatory constancy of $T_b$, but also constancy of EWL that we hypothesize may reflect a hygroregulatory response to a heliox environment.

Ventilatory data indicate how ash-grey mice accommodate the increased $O_2$ demand associated with increased thermoregulatory heat production required to maintain homeothermy in heliox at low $T_a$. As $EO_2$ did not increase in heliox at or below thermoneutrality, mice increased $V_l$ by 1.6 times in heliox (compared to air) to accommodate their increased MR. This 1.6 times increase in $V_l$ was consistent with heliox/air ratios for $C_{wet}$ and MR of 1.6. Minor increases in $V_T$ and more substantial increases in $f_R$ were the mechanisms by which mice increased $V_l$ in heliox. Generally, small mammals increase $f_R$ rather than $V_T$ to meet increased $O_2$ demand, while larger species increase $V_T$ rather than $f_R$, presumably due to the physics and mechanics of ventilation for small vs. large species (11). It has been suggested for dogs and humans that there are some minor effects of heliox on lung volume characteristics (3, 5, 14), but our data provide no evidence of heliox influencing ventilatory variables beyond that expected to accommodate the thermoregulatory increase in MR.

At $T_a$ below thermoneutrality, heliox/air ratios for the variables $C_{wet}$, MR and $V_l$ were consistent at 1.6. For purely conductive heat loss, the heliox/air ratio is 4, it is 2.1 for convective heat loss and is assumed to be 1 for evaporative and radiative heat loss. For mammals and birds this ratio for MR does not exceed 2.6, reflecting heat loss from a combination of these avenues (37, 42). The ratio is usually higher for well-insulated species (conductive heat loss is more important) and lower for poorly insulated species (convective heat loss is more important). Heat lost by evaporation and radiation can further reduce the heliox/air ratio for $C_{wet}$ and MR to <2 e.g., 1.4 for hairless mice (37) and 1.6 for ash-grey mice (this study).

We are unaware of any data examining the effect of heliox on EWL for small birds or mammals. Despite the common assumption that a heliox atmosphere should not affect EHL (19, 23, 37), water vapor diffuses 2.3 times more rapidly in heliox compared with air (21, 32, 33), so we would expect enhanced EWL from ash-grey mice in a heliox atmosphere due to an increase in cutaneous EWL, and also due to increased EWL from increased $V_l$. Yamaguchi et al. (57) showed the expected effect of increased diffusion in heliox (but confounded with hyperbaria) on cutaneous EWL of humans, and we confirmed the physical effect of enhanced EWL in heliox for our respirometry system by measuring a significant increase in EWL in heliox from both a perforated plastic vial and dead mice. The heliox effect was greater for furred mouse carcasses than for the plastic vial, presumably because the fur reduced convective effects, resulting in proportionally greater diffusive effects. These measurements demonstrated that there is a measurable increase in EWL in heliox in the absence of physiological processes, and therefore, EWL should increase in a heliox atmosphere for live mice. Our sensitivity analysis suggests that we would just be able to measure even the smallest predicted increase in EWL of mice in heliox, which would occur if the only effect was due to enhanced EWL due to increased $V_l$ for the most extreme likely combination of respiratory/cutaneous partitioning (where 80% of total EWL is cutaneous) with our humidity probes (accuracy 1%, measuring a 2% increase in RH if the only change is a 1.6 times increase in the 20% respiratory component of EWL). Other combinations of cutaneous/respiratory partitioning and diffusive heliox effects predict greater differences in EWL between air and heliox, which we would certainly be able to measure. Our repeated-measures approach for data analysis would statistically detect any consistent (i.e., all individuals increasing in heliox) measured effect even if very small. So, observation of no significant effect of heliox on EWL at or below thermoneutrality is, therefore, completely unexpected considering the physical effects of heliox on diffusion and the other expected physiological responses of mice to $T_a$ and heliox. Why then did EWL not increase in heliox?

That live ash-grey mice acutely regulate their EWL to some minimal rate at $T_a \approx$ thermoneutrality, supports previous observations of EWL constancy under conditions of varying RH or $\Delta wvp$ for various species of birds and mammals (e.g., 12, 53). Maintaining EWL constant clearly requires both sensor input and effector control. There is as yet no clear understanding of the role of constancy of EWL, let alone sensory mechanisms or control systems to explain these observations. It is possible the function of maintaining EWL constant is to avoid thermoregulatory impacts of changes in evaporative heat loss (53), so thermoregulatory sensory systems may be a fruitful avenue of investigation. Mechanisms whereby mammals and birds alter their EWL (to acclimate to chronic change in water availability) have been identified for both respiratory and cutaneous components of EWL (e.g., 43–45), and these chronic control systems may have more acute functions for EWL control.

Changes in nasal counter-current heat and water exchange could potentially modulate respiratory EWL, and contribute to total EWL constancy for ash-grey mice. Expired air temperature varies considerably for different mammals and birds (e.g., 39) and with $T_a$ and RH (15), so it could modulate respiratory
EWL. RH of expired air is generally assumed to be 100% (saturated) at expired air temperature (e.g., 10, 20), but has been measured at less than 100% for camels (40), ostrich (55), sheep (20), and humans (46), so desaturation of expired air is one conceivable mechanism for reducing respiratory EWL.

EWL could also be modulated by changes in cutaneous EWL. In fact, comparison of our sensitivity analysis with our data suggests that it is changes in cutaneous EWL that most likely contribute to EWL constancy. We know from our measurements of dead mice and plastic vials that there is an effect of heliox on EWL, confirming a diffusive component for EWL in the absence of physiological processes. Our results for live mice conform most closely to the situation of 80% cutaneous water loss and no direct heliox effect, suggesting that physiological responses that maintain constancy of EWL under perturbing conditions most likely involve control of cutaneous EWL. For arid-adapted kangaroo rats, acclimation to a more mesic laboratory environment resulted in an increase in cutaneous but not respiratory EWL, suggesting that cutaneous EWL (which is 56–71% of total EWL) is more plastic and is reduced to conserve water under conditions that facilitate evaporation (45). Decreased skin temperature would reduce the Δvvp driving cutaneous EWL, as would increased epidermal resistance to evaporation. Skin temperature changes with Ta and RH (e.g., 15), so it could be a mechanism to modulate cutaneous EWL. Ceramide levels in the epidermis largely determine skin resistance to EWL, and contribute to environmental differences in cutaneous water loss that, in turn, relate to ecological correlates of total evaporative water loss in birds (6). Ceramide levels are plastic and are associated with a decrease in EWL in response to medium-term (weeks) acclimation to low RH (31). So, although there is currently no evidence that these ceramide-associated changes in EWL can occur acutely over a period of hours (as would be required to account for adjustments to evaporative conditions within a metabolic chamber), cutaneous EWL can increase rapidly for birds in response to high Ta thermoregulatory stress (18). It is possible that increased skin resistance contributes to acute maintenance of EWL constancy, reflecting the current view of the “persistent metabolic activity” of the stratum corneum (17).

Perspectives and Significance

We present here clear evidence that ash-grey mice can acutely control their EWL under perturbing environmental conditions (heliox). On the basis of these observations, we hypothesize that hygroregulation at and below thermoneutrality may be an important aspect of the physiology of small mammals. Our findings potentially change our understanding of fundamental homeostatic mechanisms, by considering that “insensible” EWL may be a physiological process subject to regulation and not a passive consequence of the environment. This finding has implications for our understanding of the water balance of mammals, revealing how they interact with and function in their current and future environments. Determining the sensory and control systems involved with maintaining EWL constant will be important directions for future research.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: C.E.C. and P.C.W. conception and design of research; C.E.C. and P.C.W. performed experiments; C.E.C. and P.C.W. analyzed data; C.E.C. and P.C.W. interpreted results of experiments; C.E.C. and P.C.W. prepared figures; C.E.C. and P.C.W. drafted manuscript; C.E.C. and P.C.W. edited and revised manuscript; C.E.C. and P.C.W. approved final version of manuscript.

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