Does acclimation to contrasting atmospheric humidities affect evaporative water loss in the South American subterranean rodent *Ctenomys talarum*?

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Water conservation is challenging for terrestrial life since water is continuously lost through respiration, excretion, and cutaneous evaporation. Total evaporative water loss (TEWL) is an important component of the water budget. In general, TEWL is mainly determined by biophysical mechanisms, such as ambient temperature (T_a) and humidity. However, it has also been suggested that TEWL can be actively regulated in the short term to confront environmental conditions and be further modified by development and acclimation. Thus, regulation of TEWL might be complex, especially in semifossorial species, which continuously meet contrasting conditions at the surface. We evaluated the influence of acclimation to different ambient humidities on TEWL and associated metabolic parameters in the subterranean rodent *Ctenomys talarum*. We found that changing humidity acclimation conditions may not elicit modifications on TEWL. Both the water vapor–saturated burrows and the stability in TEWL at different humidities would lead to overheating problems at high ambient temperatures. Then, other forms of heat loss such as dry conductance may be enhanced. Fossoriality apparently evolved as a way of surviving increasing aridity conditions; therefore, other behavioral adjustments might be employed to counteract the high humidity within burrows.

Un gran desafío para la vida terrestre es la conservación de agua, que es continuamente eliminada a través de la respiración, excreción y evaporación cutánea. La pérdida de agua total por evaporación (TEWL) es una componente muy importante del presupuesto energético. La TEWL se encuentra principalmente determinada por mecanismos biofísicos, como la temperatura (T_a) y humedad, pero también podría ser regulada a corto plazo para afrontar variaciones ambientales, y modificada durante el desarrollo y la aclimatación. Así, la regulación de la TEWL sería compleja, especialmente en especies semifosoriales, que continuamente experimentan condiciones contrastantes sobre la superficie. Evaluamos la influencia de la aclimatación a diferentes humedades sobre la TEWL y parámetros metabólicos asociados en el roedor subterráneo *Ctenomys talarum*. Encontramos que las variaciones en la humedad de aclimatación no serían suficientes para desencadenar modificaciones en la TEWL. Si bien la característica saturación de vapor de agua en las cuevas y la constante TEWL a diferentes humedades de aclimatación conllevan problemas de sobrecalentamiento a altas T_as, otras vías de pérdida de calor serían magnificadas, tal como la conductancia térmica seca. Considerando que la fosorialidad aparentemente evolucionó como forma de supervivencia frente a la aridez creciente, otros ajustes comportamentales serían empleados para contrarrestar la alta humedad dentro de las cuevas.

Key words: *Ctenomys talarum*, evaporative heat loss, humidity regime, resting metabolic rate, subterranean rodents, total evaporative water loss

Water conservation is challenging for terrestrial life, especially, but not exclusively, in species that live in hot and dry environments. Individuals continuously expend energy to maintain physiological processes associated with water balance. They not only consume exogenous water but also produce it through metabolic processes. On the other hand, water is lost through

respiration, excretion, and cutaneous evaporation. Total evaporative water loss (TEWL) is an important component of the water budget and comprises both cutaneous and respiratory water loss, the former constituting more than 70% of total evaporation (Williams and Tieleman 2005; Cooper and Withers 2014). However, the relative contribution of respiratory and cutaneous loss to TEWL can vary (Baudinette 1972; Hillenius 1992; Williams and Tieleman 2005; Withers et al. 2012; Minnaar et al. 2014). In subterranean rodents, sweat glands are absent and cutaneous evaporation may occur through diffusion depending on skin characteristics and water vapor pressure gradient between body and surrounding air (McNab 1966; Buffenstein and Yahav 1991). TEWL may depend not only on morphological and physiological adaptations but also on ambient variables (Baudinette 1972; Zhu et al. 2008; Minnaar et al. 2014). However, several studies refute the idea that TEWL is determined basically by biophysical mechanisms (Withers and Cooper 2014), suggesting that TEWL can be actively regulated in the short term to confront environmental conditions and further modified by development and acclimation (Withers et al. 1979; Tracy and Walsberg 2001a; Cooper and Withers 2014; Withers and Cooper 2014). Short-term responses related to diminishing TEWL have been observed, for example, such as a reduction in the frequency of lung ventilation in hibernating bats in response to ambient temperature (Thomas and Geiser 1997) or in the permeability of the stratum corneum of sparrows when acclimated to dry air (Muñoz-Garcia et al. 2007).

Regulation of TEWL might be complex, as it may involve the interaction between different ambient conditions, such as humidity and ambient temperature (T_a), and behavioral mechanisms. In some species, ambient humidity and T_a are important influences on TEWL (Baudinette 1972; Zhu et al. 2008; Minnaar et al. 2014), while in others, evaporation can be actively regulated (Cooper and Withers 2008; Withers and Cooper 2014). In the brushtail possum *Trichosurus vulpecula*, it was observed that TEWL is negatively related to humidity at T_a of 30°C but is independent at 25°C. This occurrence is probably associated with postural differences (relative humidities analyzed: from 5% to 85%—Cooper and Withers 2008). By changing posture, individuals can reduce cutaneous water loss and generate an exceptional high humidity microenvironment where respiration may reduce water loss (Bintz and Roesbery 1978). Furthermore, Cooper and Withers (2014) found that the ash-gray mouse *Pseudomys albocinereus* can control TEWL, even when thermogenic mechanisms are maximally elicited because of an increment in heat loss. On the other hand, TEWL contributes to heat loss by evaporation (EHL), in cases where heat production (HP) exceeds the possibility of its dissipation by conductance. This may depend on individuals' metabolic rate, temperature, and activity (Huynh et al. 2007; Zhu et al. 2008).

The burrow represents a singular medium in which individuals live, to some degree, under high humidity and stable temperatures (see Baudinette 1972; Burda et al. 2007). Such microenvironments are interesting for assessing the influence of abiotic variables on EWL. For example, in the California ground squirrel *Otospermophilus beecheyi*, ambient humidity has a negative direct relationship with EWL but no effect on resting metabolic rate (RMR) or body temperature (T_b). Moreover, the microenvironment of burrows remains quite stable despite daily ambient fluctuation at the surface in humidity and T_a. These parameters may not significantly affect an individual's physiology; they support the idea that behavioral adaptations have an important role in individual homeostasis (Baudinette 1972).

Water balance, particularly TEWL, has been broadly studied for desert species (Schmidt-Nielsen and Schmidt-Nielsen 1950a; Hinds and MacMillen 1985; Cortés et al. 2000a; Diaz and Cortés 2003) and some burrowing mammals (Chew 1951; Baudinette 1972). However, little work has been done on species that inhabit permanently closed tunnel systems (see Buffenstein and Yahav 1991) or in species that live in tunnel systems but experience surface humidity and temperature fluctuations during their regular exits to gather food (see Cortés et al. 2000b).

Ctenomys talarum (tuco-tucos) is a solitary subterranean rodent from the coastal grassland of Buenos Aires (Comparatore et al. 1991). Its burrows comprise a system of closed galleries, characterized by a main tunnel connected with several lateral branches, most of which are connected to the outside by a few entrances that are generally closed (Antinuchi and Busch 1992; Vassallo 2006). The microclimate of such burrows exhibits low concentrations of O_2, high levels of CO_2, and high humidity (McNab 1966). Tuco-tucos live in a mesic environment, inside water vapor–saturated burrows, and may experience atmospheric water variations as they have short bouts to the surface to gather food (expending < 1% of the day aboveground—del Valle et al. 2001; Vassallo 2006). Individuals also experience surface atmospheric conditions during the dispersal period, which occurs above ground at > 45 days of age (Malizia et al. 1995). This behavior consists of short exploratory behaviors on the surface and the construction of a simple burrow within 2 days, which will be regularly modified (Vassallo 2006). Also, during the reproductive period, tuco-tucos experience atmospheric changes when exiting burrows to find mates, although courtship and copulation occur underground (Zenuto et al. 2002). Regarding feeding ecology, *C. talarum* explores briefly on the surface, foraging mostly on aerial parts of grasses such as *Poa bonariensis*, *Bromus uniloides*, and *Panicum racemosum* (Comparatore et al. 1995; del Valle et al. 2001). This species does not drink free water but obtains exogenous water only from food (Zenuto et al. 2001; Baldo et al. 2014). However, the water content of most items varies greatly throughout the year (e.g., from 30% to 90% for *P. racemosum*—Fanjul et al. 2006). Furthermore, morphological and physiological characteristics of this species are similar to those observed in other subterranean rodents, highlighting their low RMR, compared to surface rodents of similar size (see Luna et al. 2009). This would contribute to a lower energy requirement and water exchange ratio, also diminishing the necessity for heat dissipation (Buffenstein 2000).

Thus, the aim of this study was to evaluate the effect of short-term acclimation to low and high ambient humidity (H)

on TEWL, and associated metabolic parameters, in the subterranean rodent *C. talarum*. We expected to observe a higher TEWL in a change from low to high humidity acclimation (similar to burrows), when compared to dry air (i.e., maximal TEWL), as the animal may not display strategies for reducing water loss during breathing in a high humidity atmosphere. On the other hand, TEWL will probably be reduced if acclimation to a humid microenvironment goes from high to low, as it may compromise the animal's water balance.

MATERIALS AND METHODS

Animals and maintenance.—Adult males ($n = 14$) of *C. talarum* were livetrapped in Mar de Cobo locality, Buenos Aires. After that, animals were transported to the laboratory, housed in individual cages with wood shavings and a hiding place, consisting of a one-half terracotta flowerpot. Individuals were fed ad libitum with sweet potato, lettuce, corn, and sunflower seeds. As these rodents do not drink free water, it was not provided (Zenuto et al. 2001). Photoperiod (12L:12D) and ambient temperature ($T_a = 25°C$; corresponding to thermoneutrality—Busch 1989) were controlled and maintained constant during the entire experiment. Animals were housed and maintained in accordance with the guidelines for the capture, handling, and maintenance in captivity of mammals of the American Society of Mammalogists (Sikes et al. 2011) and current laws of Argentina.

Ambient humidity effect.—To test the effect of ambient humidity (H) acclimation on TEWL at thermoneutrality (Busch 1989), captured individuals were randomly assigned to different experimental groups. One group was maintained at high humidity condition (99% relative humidity [RH] at $T_a = 25°C$; equivalent to an absolute humidity of 0.023 kg/m³; $n = 7$), whereas the other group was maintained at low humidity (50–60% RH at $T_a = 25°C$; equivalent to 0.012–0.014 kg/m³; $n = 7$) for the same period. Ambient humidity was controlled by regulating ventilation of the cages that were in the room and was permanently monitored to maintain stability. Ventilation was modified by covering/uncovering the cages: closed cages retained humidity, had elevated CO_2, and low O_2 concentration, resembling the natural conditions of the burrows of *C. talarum* ($18.96 \pm 1.08\%$ O_2; $2.40 \pm 0.96\%$ CO_2; $94.55 \pm 5.30\%$ RH at $T_a = 26.5 \pm 1.56°C$ in summer, $72.05 \pm 6.58\%$ RH at $T_a = 6.35 \pm 0.78°C$ in winter; M. B. Baldo, IIMyC, CONICET-UNMdP, pers. comm.). Opened (uncovered) cages presented low humidity, high concentrations of O_2, and low CO_2, simulating aboveground atmospheric conditions in nature. The acclimation period lasted 25 days, based on previous studies on the same species (Perissinotti et al. 2009). After the acclimation period at high or low H, individuals were changed to the opposite experimental condition. Thus, animals that were acclimated to high H, experienced the low H condition (referred thereafter as group High → Low), and animals acclimated to low H were transferred to a high H regimen (group Low → High). After initial testing (day 0), animals were placed in the opposite condition and were retested at days 3, 7, and 11. After this period, we conducted a final test between days 21 and 30 (day > 21).

Physiological measurements.—Total evaporative water loss and RMR were estimated at thermoneutrality through respirometric techniques (Lighton 2008). Resting metabolism was obtained from O_2 consumption, which was measured using a computerized positive pressure open-flow respirometry system (Sable Systems, Las Vegas, Nevada). Animals were individually placed in a chamber (1.8 liters) which received air at 1.4 l/min from a flowmeter (Side-Trak Sierra model 830/840; Sierra Instruments, Monterey, California). Air passed through a CO_2 absorbent (self-indicating soda lime IQB; Laboratories IQB, Quilmes, Argentina) and water scrubber (Drierite; W.A. Hammond Drierite Co. Ltd., Xenia, Ohio) before going through the chamber. Excurrent air from the chamber was subsampled at 130 ± 10 ml/min. It was passed through a CO_2 analyzer (CA-10; Sable Systems, Las Vegas, Nevada), to determine CO_2 production. Then, the air was passed through IQB and Drierite, before being analyzed by an O_2 analyzer (FC-1B; Sable Systems, Las Vegas, Nevada) every 0.5 s by Expedata - PC program (Sable Systems). Baseline-corrected data of O_2 consumption and of CO_2 production were converted into ml of each gas using standard respirometric equations (Withers 1977). Initially, animals were placed in the chamber for 30 min, to allow the system to become stable. Oxygen consumption and CO_2 production were continuously monitored until a stable reading was attained and held for at least 15 min. After that, a small column containing a water absorbent (Drierite) was connected for 30 min to the system, immediately after the CO_2 analyzer. As the column was weighed before (w_1) and after (w_2) the connection, TEWL was determined gravimetrically (see Bernstein et al. 1977 for a validation of this technique compared to open-flow respirometry). A total experimental trial lasted almost 2 h. As RH can affect the value of TEWL during respirometric trials (Withers et al. 1979), and we wanted to determine the maximal capacity of individuals to reduce evaporation, the metabolic chamber was flushed with dry air and the value of TEWL obtained was considered as the maximal TEWL at the measured temperature. Data were discarded when individuals urinated within the chamber. In this case, the chamber was cleaned and data collection was repeated. Body mass (M) was measured before each experimental set (scale model FX-3000, ± 0.01 g; A&D Company Limited, San Jose, California), whereas body temperature was measured as rectal temperature, with a YSI probe (model 93k73545-402) connected to a Cole-Parmer thermistor meter (model 8402-10; Cole-Parmer Instrument Company, Vernon Hills, Illinois) after each respirometric measurement. Since in this species neither a circadian O_2 consumption nor a locomotor activity rhythm was observed (Luna et al. 2000; Meroi et al. 2014), all experimental trials were conducted between 0800 and 1700 h, at thermoneutrality (25°C—Busch 1989). The respiratory quotient (RQ) was determined as the ratio between CO_2 production and O_2 consumption, to determine potential differences in substrate catabolism (Schmidt-Nielsen and Schmidt-Nielsen 1950b). Thermal conductance was computed at thermoneutrality, as $C = RMR/(T_b - T_a)$, where T_b is body temperature after RMR estimation (Seymour et al. 1998). Additionally, other parameters were obtained, as metabolic water production (MWP = RMR * 0.62 mg H_2O/ml

O$_2$—Schmidt-Nielsen and Schmidt-Nielsen 1950b; MacMillen and Hinds 1983), evaporative heat loss (EHL = TEWL * 0.603 cal/mg H$_2$O; see Stitt 1976; Huynh et al. 2007), and heat production (HP = RMR * 5.011 cal/ml O$_2$; see Haim et al. 2008).

Statistical analyses.—All data were expressed as mean ± *SE*. The Student's *t*-test was used to determine differences between body mass at capture and at day 0, and to compare body mass after acclimation between both humidity regime groups. A repeated measures analysis of covariance (ANCOVA) was used to test for no difference among days (within-subject effect) and experimental groups (High → Low versus Low → High; between-subject effect) for TEWL, RMR, MWP, EHL, and HP between experimental condition and among days. In all cases, body mass was used as a covariate. A repeated measures analysis of variance (ANOVA) was performed to analyze differences in T_b, thermal conductance, RQ, and parameters obtained by the combination of other variables (MWP/TEWL, EHL/HP). Normality and homoscedasticity of variance were analyzed using Levene's test (Zar 2010) before applying the repeated measures ANCOVAs and ANOVAs. TEWL of *C. talarum* was compared with the prediction from standard allometric equations known for mesic rodents (Cortés et al. 2000a). The observed data were considered to conform to the predictions of the equation if falling within the 95% prediction confidence limits (Cooper and Withers 2006).

RESULTS

Body mass.—Body mass of individuals did not vary between the day of capture and day 0 (paired *t*-test, $t_{13} = -1.07$, $P = 0.31$). Additionally, no differences were found in body mass of individuals after humidity acclimation in the Low → High group (paired *t*-test, $t_6 = -1.62$, $P = 0.16$). However, individuals of the High → Low group showed a small increment in their body mass after the acclimation period (146.10 ± 6.61 g to 169.12 ± 6.40 g; paired *t*-test, $t_6 = -7.01$, $P < 0.01$).

Evaporative water loss and energetic parameters.—Evaporative water loss was similar between conditions (repeated measures ANCOVA, $F_{1,7} = 1.19$, $P = 0.31$). Also, TEWL was similar between experimental days ($F_{4,24} = 1.64$, $P = 0.19$). Moreover, the interaction between repeated factor and treatment was also similar ($F_{4,24} = 0.77$, $P = 0.56$; see Fig. 1).

No differences were observed for RMR either between groups (repeated measures ANCOVA, $F_{1,7} < 0.01$, $P = 0.98$) or among days ($F_{4,28} = 0.42$, $P = 0.80$) or their interaction ($F_{4,28} = 0.38$, $P = 0.82$). Similar results were found for thermal conductance (repeated measures ANOVA, $F_{1,12} = 0.71$, $P = 0.42$; day, $F_{4,48} = 1.07$, $P = 0.38$; interaction, $F_{4,48} = 1.95$, $P = 0.12$). Body temperature was higher in the High → Low group than the Low → High group (repeated measures ANOVA, $F_{1,12} = 11.22$, $P = 0.01$), but not among days or their interaction (respectively, $F_{4,48} = 2.06$, $P = 0.11$; $F_{4,48} = 0.68$, $P = 0.59$). RQ was also higher for the High → Low condition than the contrasting group (repeated measures ANOVA, $F_{1,12} = 17.09$, $P = 0.01$), but not days ($F_{4,48} = 0.83$, $P = 0.51$) or their interaction ($F_{4,48} = 0.38$, $P = 0.82$; see Table 1).
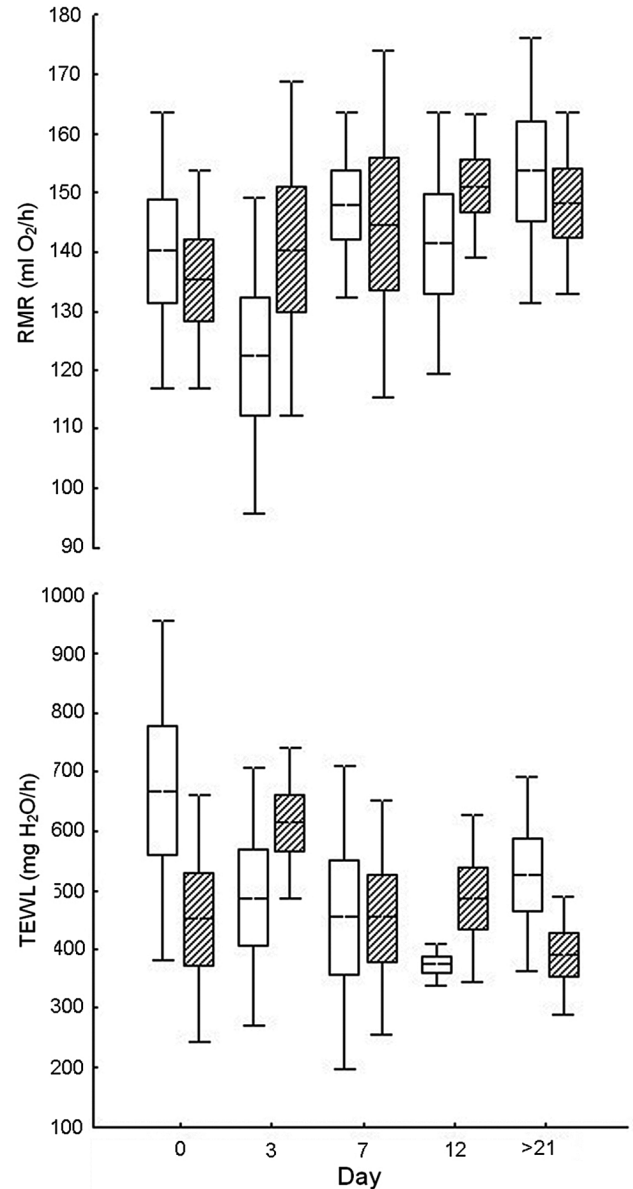

Fig. 1.—Resting metabolic rate (RMR) and total evaporative water loss (TEWL) in *Ctenomys talarum* individuals in both experimental groups: High → Low (acclimated at high ambient H and transferred to a low H regime; white) and Low → High (acclimated at low ambient H and transferred to a high H regime; shaded). Middle point: mean; box value: *SE*; whisker value: *SD*.

Other physiological parameters.—No other variables were found to be affected by humidity regime or acclimation period. Thus, similar values were obtained for MWP among days and experimental conditions (repeated measures ANCOVA, $F_{1,7} < 0.01$, $P = 0.99$; day, $F_{4,28} = 0.36$, $P = 0.84$; interaction, $F_{4,28} = 0.41$, $P = 0.80$), HP ($F_{1,7} = 0.03$, $P = 0.87$; day, $F_{4,28} = 0.31$, $P = 0.87$; interaction, $F_{4,28} = 0.71$, $P = 0.59$), and EHL ($F_{1,7} = 1.19$, $P = 0.31$; day, $F_{4,28} = 1.64$, $P = 0.19$; interaction, $F_{4,28} = 0.77$, $P = 0.56$; see Table 2). Ratios differed among certain days but were similar when taking into account the interaction between groups and days (repeated measures ANOVA, MWP/TEWL, $F_{1,12} = 0.06$, $P = 0.82$; days,

Table 1.—Body mass, respiratory quotient (RQ), body temperature (T_b), and thermal conductance (*C*) in *Ctenomys talarum* individuals in both experimental groups: High → Low (acclimated at high ambient H and transferred to a low H regime) and Low → High (acclimated at low ambient H and transferred to a high H regime). Values are expressed as Mean ± *SE*.

		Day 0	Day 3	Day 7	Day 11	Day > 21
Body mass (g)	High → Low	146.1 ± 6.62	150.46 ± 6.84	155.31 ± 7.01	161.44 ± 7.15	169.12 ± 6.40
	Low → High	162.41 ± 11.04	160.71 ± 10.30	159.35 ± 11.08	164.43 ± 10.49	167.61 ± 10.34
RQ[a]	High → Low	0.92 ± 0.03	0.90 ± 0.03	0.96 ± 0.04	0.97 ± 0.03	0.92 ± 0.04
	Low → High	0.86 ± 0.05	0.82 ± 0.03	0.83 ± 0.03	0.88 ± 0.04	0.87 ± 0.04
T_b (°C)[a]	High → Low	35.9 ± 0.2	35.9 ± 0.2	36.0 ± 0.2	35.8 ± 0.2	35.6 ± 0.1
	Low → High	35.5 ± 0.2	35.1 ± 0.2	35.0 ± 0.3	35.0 ± 0.3	34.9 ± 0.3
C (ml O$_2$/°C h)	High → Low	12.90 ± 0.86	11.36 ± 0.87	13.47 ± 0.40	13.12 ± 0.78	14.58 ± 0.74
	Low → High	12.99 ± 0.81	13.93 ± 0.90	14.42 ± 1.08	15.12 ± 0.37	15.07 ± 0.60

[a]T_b and RQ were different only between experimental groups (both, $P = 0.01$; see "Results").

Table 2.—Calculated evaporative and metabolic parameters in *Ctenomys talarum* individuals in both experimental groups: High → Low (acclimated at high ambient H and transferred to a low H regime) and Low → High (acclimated at low ambient H and transferred to a high H regime). MWP: metabolic water production (RMR * 0.62 mg H$_2$O/ml O$_2$); HP: heat production (RMR * 5.011 cal/ml O$_2$); EHL: evaporative heat loss (TEWL * 0.603 cal/mg H$_2$O). Values are expressed as Mean ± *SE*.

		Day 0	Day 3	Day 7	Day 11	Day > 21
MWP (mg H$_2$O/h)	High → Low	86.93 ± 5.47	76.73 ± 6.33	91.76 ± 3.66	87.71 ± 5.16	95.29 ± 5.22
	Low → High	83.92 ± 4.32	87.11 ± 6.59	89.73 ± 6.86	93.70 ± 2.80	91.95 ± 3.60
MWP/TEWL	High → Low	0.16 ± 0.03	0.17 ± 0.02	0.26 ± 0.05	0.24 ± 0.02	0.20 ± 0.03
	Low → High	0.22 ± 0.03	0.15 ± 0.02	0.23 ± 0.04	0.21 ± 0.03	0.25 ± 0.02
HP (cal/h)	High → Low	702.51 ± 44.20	620.12 ± 51.19	741.58 ± 29.60	708.87 ± 41.71	770.10 ± 42.22
	Low → High	678.22 ± 34.89	703.97 ± 53.27	725.13 ± 55.44	757.21 ± 22.62	743.05 ± 29.12
EHL (cal/h)	High → Low	403.1 ± 65.30	297.6 ± 50.50	273.82 ± 58.59	225.47 ± 8.10	317.76 ± 37.74
	Low → High	272.49 ± 47.61	370.58 ± 28.90	272.98 ± 45.16	293.55 ± 32.26	235.01 ± 22.78
EHL/HP	High → Low	0.59 ± 0.10	0.48 ± 0.07	0.36 ± 0.07	0.33 ± 0.03	0.46 ± 0.07
	Low → High	0.41 ± 0.08	0.55 ± 0.05	0.39 ± 0.07	0.39 ± 0.04	0.31 ± 0.03

$F_{4,48} = 3.21$, $P = 0.02$; interaction, $F_{4,48} = 1.59$, $P = 0.19$; EHL/HP, $F_{1,12} = 0.27$, $P = 0.61$; day, $F_{4,48} = 3.81$, $P = 0.01$; interaction, $F_{4,48} = 2.59$, $P = 0.05$; Table 2).

Comparing TEWL for *C. talarum* (log transformed) with the standard allometric equations of Cortés et al. (2000a), it was found that tuco-tucos present a relatively high evaporative rate for their body mass (*C. talarum*'s data: 2.75 mg H$_2$O/h; prediction confidence limits of the regression: 1.63–2.36).

Discussion

Evaporation is known for being one of the main venues of both water loss and heat dissipation, particularly in mammals living in hot and dry habitats, and characterized by a high surface/volume ratio (Chew 1951; Cortés et al. 2000a). However, animals inhabiting mesic environments may also present adaptations to prevent water loss. Although their TEWL is relatively high compared to species from extreme climates, which present adaptations for avoiding water loss (Cortés et al. 2000a), mesic species also have the ability for balancing their water budget (Tracy and Walsberg 2001a; Bozinovic et al. 2011), even after taking into account body mass and phylogeny. Evaporative water loss is a character constrained by environmental characteristics and shows a low phylogenetic signal (Van Sant et al. 2012).

In the case of the subterranean rodent *C. talarum*, which inhabits water vapor–saturated burrows differing greatly from outside conditions, their TEWL was independent of acclimation to different humidity regimes. Thus, the effect of changing acclimation to different atmospheric humidity might not be enough to elicit an adjustment in evaporation rates (see Fig. 1). Some rodents, such as *O. beecheyi* and *Peromyscus maniculatus*, show a direct relationship between evaporation rate and deficit of water vapor pressure within a wide range of atmospheric humidity (Baudinette 1972; Edwards and Haines 1978). On the other hand, the marsupial *T. vulpecula*'s TEWL is affected by H > 63% RH at 25°C or by humidities of 26–92% RH at 30°C (Cooper and Withers 2008). Differences have also been observed in the response of metabolic rate to H: in *T. vulpecula*, RMR increases at decreasing RH at T_a of 30°C (Cooper and Withers 2008), while in *S. beecheyi*, metabolic rate is independent of H (Baudinette 1972). On the other hand, constant TEWL has been observed for other mammals, which may not be a passive process (Cooper and Withers 2014). Also, the independence of TEWL from the difference in water vapor pressure between individual and ambient is more common in endotherms than had been thought (Withers and Cooper 2014).

A component of TEWL is respiratory water loss, which depends not only but greatly on the water vapor pressure gradient among individuals and the surrounding atmosphere (Gates 2012). In the case of tuco-tucos, a modest atmospheric water gradient (i.e., inside burrows) would almost prevent evaporation's contributing to water conservation, as they would be exhaling water-saturated air. Even though soil porosity and burrow

ventilation considerably affect air humidity within burrows (Walsberg 2000), preliminary measurements indicate that a high atmospheric humidity is maintained inside the tuco-tuco's subterranean tunnels (see Baldo et al. 2015). This may be provided by the soil water content in this mesic area (3.6–5% of soil mass—Malizia et al. 1991; Antinuchi and Busch 1992) and also the high rates of TEWL of *C. talarum* individuals compared to xeric rodents (Cortés et al. 2000a; Table 3). Both characteristics result in a unique atmospheric humidity in tuco-tucos burrows, different from other desert rodents (see Walsberg 2000). Therefore, behavioral strategies such as living inside closed burrows can be enough to reduce TEWL (Chew 1951; Gettinger 1984; Cooper and Withers 2008; Gates 2012). On the other hand, a greater variation in water vapor pressure occurs on the surface, when these individuals leave burrows to forage, disperse, or reproduce. In this study, we did not detect variations in TEWL for individuals acclimated to contrasting ambient H when measurements involved dry air flux (i.e., maximal TEWL). Nevertheless, values of TEWL at saturated humidity may provide interesting information on the evaporative dynamics for tuco-tucos in their burrows. In this context, it would be important in future studies to measure evaporation rates in a water vapor–saturated air flux, in order to be able to extrapolate these values to what occurs under natural conditions (i.e., in burrows).

Indicators of water regulation efficiency have been developed in many studies, involving not only traditional parameters such as cortex/medulla ratio in kidney but also respiratory and metabolic ones (see Bozinovic and Gallardo 2006). MacMillen and Hinds (1983) proposed the MWP/TEWL ratio as a physiological indicator of water regulation efficiency, suggesting that there is a T_a at which an individual can survive on only a dry diet, without exogenous water, and this corresponds to MWP/TEWL = 1. Here, we evaluated this parameter at thermoneutrality and obtained a small value (0.16–0.22 for individuals acclimated to high and low H, respectively; day 0, see Table 2). Thus, even though individuals do not consume free water, they are expected to require the consumption of hydrated food to maintain water balance. Individuals might need to consume more food in periods in which vegetation shows a lower water content (Fanjul et al. 2006).

Evaporative water loss depends on both humidity and thermal gradients between individuals and the surrounding atmosphere (Edwards and Haines 1978) and is a venue for heat loss. This means that evaporative heat loss is a component of thermal conductance, which is a measure of heat exchange and depends not only on individuals characteristics (i.e., RMR and T_b) but also on environmental conditions (McNab 1966). As in other subterranean rodents, *C. talarum* is characterized by a low RMR; hence it is characterized by low HP and high thermal conductance in comparison with surface rodents of similar body mass (McNab 1966; see Luna et al. 2009). In this study, we did not detect variations in RMR among experimental conditions or days, suggesting that ambient H does not affect basal metabolism as was found for other rodents (Baudinette 1972; Edwards and Haines 1978). Nevertheless, it was observed that RQ and T_b differed between treatments, being higher for H → L in both cases (see Table 1). RQ is an indicator of the main type of substrate being used by an individual (Schmidt-Nielsen 1997). Though differences were observed, both groups might use glucids as the major energetic source (0.82 ≤ RQ ≤ 0.96; Table 1). However, as RQ < 1, other substrates are also being catabolized. Further analysis is needed to determine the nature and level of each contribution (Schmidt-Nielsen and Schmidt-Nielsen 1950b), principally for L → H. This group has the lower RQ, and the oxidation of lipids could be providing a greater amount of water per unit of heat. However, the difference in RQ may be a consequence of a disparity in body mass between groups and therefore in the use of energetic substrates. Nevertheless, we observed greater food consumption in the group H → L than in L → H from day 0 to day > 21 (not shown). This fact may be associated with the exposure to dryer atmospheric conditions, resulting in an elevated requirement for exogenous water consumption to maintain water balance, which in this species occurs only by food intake (Zenuto et al. 2001).

Some studies did not find variations related to ambient H (Baudinette 1972; Edwards and Haines 1978). However, others detected a slight positive correlation, probably attributed to a reduction in the possibility of evaporative cooling (Kay 1975). In the little kaluta *Dasykaluta rosamondae*, an independence of T_b was observed, and also TEWL, at T_as within the thermoneutrality range. Thus, water is conserved at low H and T_a, as no adjustment in EHL is needed to maintain normothermy (Withers and Cooper 2014). In this case, tuco-tucos showed higher values of T_b in the group acclimated to high H (Table 1); this could be a consequence of a reduced capacity for

Table 3.—Data of total evaporative water loss (TEWL) for subterranean rodents. Expected values of TEWL for xeric and mesic species were calculated using the allometric equations obtained by Cortés et al. (2000a).

Species	Acclimation at	Measurement conditions	TEWL measured (mg H$_2$O/gh)	TEWL[a] (xeric)	TEWL[b] (mesic)	References
Ctenomys talarum	99% H, 25°C[c]	Dry air, 25°C	4.55±0.64	0.75	1.22	This study
Ctenomys talarum	50–60% H, 25°C	Dry air, 25°C	2.80±0.42	0.72	1.16	This study
Ctenomys opimus	Unknown	Unknown H, 10–25°C	1.56	0.60	0.92	Diaz and Cortés (2003)
Ctenomys fulvus	60% H, 21°C[c]	60% H, 10–25°C	1.26	0.61	0.94	Cortés et al. (2000b)
Heterocephalus glaber	70–80% H, 30°C[c]	50–60% H, 31–34°C	16.59	1.25	2.34	Buffenstein and Yahav (1991)
Thomomys bottae	Unknown H, 24–31°C	Unknown H, 27.5°C	4.30	0.86	1.45	Gettinger (1984)

[a]TEWL = 5.97 M$^{-0.416}$.
[b]TEWL = 17.27 M$^{-0.532}$.
[c]Conditions similar to burrow atmosphere.

evaporative heat dissipation at high water pressure, leading to an elevation of T_b (Edwards and Haines 1978).

Finally, it is important to standardize measurements for certain interspecific comparisons, but it is also necessary to represent the animal's natural conditions. Data obtained within the range of environmental variation will allow a deeper understanding of the challenges that individuals actually face in the wild. Most studies measure maximal TEWL (i.e., at dry air), without considering natural conditions, regardless of whether animals inhabit a xeric or mesic ambient, or whether they reside on the surface or within subterranean environments. For instance, if we compare TEWL for tuco-tucos with the standard allometric equations for rodents from xeric or mesic habitats (see Cortés et al. 2000a), it shows a higher TEWL (Table 3). Interestingly, the same pattern was also found in other subterranean rodents, such as *Thomomys bottae* (see Table 3); even though TEWL values also differ among rodents of underground habits (i.e., from *Heterocephalus glaber*; see Table 3).

In conclusion, we suggest that tuco-tucos are adapted to sustain water balance by maintaining TEWL in dry air, independently of the atmospheric H of acclimation; this may compromise heat loss through evaporation. However, the low RMR in this species results both in a reduced MWP and in a low HP (see equations above). Hence, body temperature is balanced.

We demonstrated that *C. talarum* can maintain total evaporation rate independently of short-term atmospheric humidity acclimation. Thus, the high humidity within burrows and the consistent TEWL might lead to overheating problems at high T_as. However, other means of heat loss might be enhanced. Dry thermal conductance is the main avenue for heat loss at temperatures above the thermoneutral zone in *C. talarum* (Baldo et al. 2015). Therefore, behavioral adjustments, rather than more costly changes in physiological mechanisms, might be used to counteract the high humidity observed within burrows. Fossoriality apparently evolved as a way of adapting to new, increasingly arid conditions (Nevo 1979). Nevertheless, further studies are needed to evaluate intraspecific phenotypic variations along an environmental gradient (Tracy and Walsberg 2001a, 2001b; Bozinovic et al. 2011).

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