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Effect of Ventilated Caging on Water Intake and Loss in 4 Strains of Laboratory Mice

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Food availability, temperature, humidity, strain, and caging type all affect water consumption by mice. Measurement of transepidermal water loss (TEWL) is a new technique for the quantification of water turnover in mice. To understand water turnover in common strains of adult mice, male and female SCID, SKH, C57BL/6, and FVB mice were housed in same-sex groups of 5 animals in static cages or IVC. Body weight, TEWL, urine osmolality, and water consumption of mice and intracage temperature and humidity were measured every 48 h for comparison. Static cages were monitored for 7 d and IVC for 14 d before cage change. Female SCID, FVB, and C57 mice drank less water than did their male counterparts. Male and female SCID, SKH, and FVB mice in IVC drank less water and had higher urine osmolality than did those in static cages. In SCID and SKH mice, TEWL paralleled water consumption. C57 mice in static cages drank less water, had lower urine osmolality, and had less TEWL than did those in IVC. Temperature and humidity within the cage was higher than the macroenvironmental levels for all housing conditions, mouse strains, and sexes. Temperatures within IVC ranged from 76.6 to 81.4 °F compared with 69 ± 0.4 °F in the room. Humidity within IVC ranged from 68% to 79% compared with 27.0% ± 2.7% within the room. These data demonstrate that mouse strain and housing conditions significantly influence water balance and indicate that macroenvironmental measurements do not always reflect the intracage environment.

Abbreviation: TEWL, Transepidermal water loss

The ability to regulate water balance is essential for life. In mice, water intake is affected by food availability, environmental temperature and humidity,[18] mouse strain,[2] and type of caging.[32] Interstrain differences in water consumption reportedly vary from 3.9 to 8 mL per mouse per day in static open top cages,[2] and standard recommendations regarding water consumption vary from 3.75[46] to 9.5[2] mL per 25-g mouse. These recommendations are based on water intake by different strains of mice housed individually in open-top cages with wire grids.[2,5] The administration of experimental compounds and medical treatments in drinking water is standard in the laboratory animal community. Accurate dosage of drugs administered in the water is confounded by the number of mice in a cage. To maintain drug efficacy, treated water usually has to be changed frequently, which requires increased personnel time and can waste expensive experimental compounds. Conversely, attempts to minimize wastage may result in failure to provide sufficient water, resulting in harm to the animal and regulatory compliance concerns. Despite the importance of water consumption and the use of water as a vehicle for experimental manipulation and therapeutic treatment, little is known about the effect of ventilated caging on water turnover in mice. An improved understanding of how water is metabolized in mice housed under different conditions likely will alleviate some of these concerns.

There are 2 major routes of water loss in mammals. Sensible water loss is defined as water loss through urine and sweat, because this loss is physiologically regulated. Sensible water loss primarily occurs through urine in mice, given that water loss through sweat is minimal due to a lack of sweat glands (except for the footpad).[18] Insensible water loss occurs through the respiratory tract and skin and is generally independent of physiologic state.[12] Transepidermal water loss (TEWL) represents a significant portion of insensible water loss for mice and is therefore a major component of water balance, comparable in daily volume to urine.[12] Increased insensible water loss in mice contributes to the development of dehydration during 'old age' (14 to 24 mo),[12] and mouse skin has been suggested to adjust to maintain body water content according to environmental conditions.[18,41] TEWL can be quantified directly by using an evaporimeter, which measures the rate of water exchange across the dermis. The evaporimeter probe contains an open cylinder with paired sensors, which are placed perpendicular to the skin surface. The probe measures humidity and temperature at each sensor and computes 2 separate vapor pressures. The difference between the vapor pressures at these 2 points is used to calculate evaporative water loss from the skin.[15] TEWL measurements have been used as a reliable technique for quantification of skin function.[15,29,48] However, this technique has not been systematically applied to understanding water turnover in mice.

The high airflow in IVC has been proposed to cause dehydration[4] and cold stress[3,10] in mice. The effect of IVC housing on mouse behavior has been studied in an attempt to quantify stress and animal wellbeing.[7,25,52] A variety of housing systems have identified intracage differences in ammonia levels,[7,32,38,39,52] carbon dioxide,[52] and particulate levels[39] as important considerations for animal wellbeing. Although some of these studies report water consumption as an ancillary indication of animal health,[7,32,52] the influences of strain and housing type on water turnover have not been compared systematically.

The current studies used 4 common strains of hairless and furred mice to determine the effects of fur and strain on water turnover under IVC conditions. SKH (hairless immunocompetent), SCID nude (hairless immunocompromised), C57BL/6, and FVB mice were housed in microisolation cages under static or IVC conditions. Male and female mice were used to

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determine the influence of sex on water metabolism. To determine water intake and insensible and sensible water loss, we measured water consumption, TEWL, body weight, and urine osmolality. Intracage and room humidity were recorded every 48 h. We hypothesized that mice housed under IVC conditions would require more drinking water, have more concentrated urine, and have higher TEWL levels as compared with mice in static cages. Likewise we predicted that hairless mice would have greater water loss via TEWL than would furred strains. An increased understanding of the influence of housing on water turnover in commonly used strains of mice is important information that should be considered when interpreting data or when providing treatments in drinking water.

Materials and Methods

Animals. Male and female SCID (hairless SHC, strain code 488), SKH (strain code 477), FVB (strain code 207), and C57 (strain code 027) mice were obtained at 8 wk of age from Charles River Laboratories (Wilmington, MA). Mice were tested and found to be free from infections with cilia-associated respiratory bacillus, *Citrobacter rodentia*, *Clostridium piliforme*, *Corynebacterium kutscheri*, ectromelia virus, *Encephalitozoon cuniculi*, lymphocytic choriomeningitis virus, minute virus of mice, mouse adenovirus, mouse coronavirus, Theiler murine encephalomyelitis virus, mouse hepatitis virus, mouse parvovirus, mouse rotavirus, mouse thymic virus, *Mycoplasma pulmonis*, *Myobia musculi*, *Pasteurella pneumotropica*, pneumonia virus of mice, polyoma virus, reovirus 3, *Salmonella* spp., *Streptococcus pneumoniae*, *Helicobacter* spp., and endo- and ectoparasites. All studies were approved by The Ohio State University IACUC and were conducted in AAALAC-accredited facilities.

Housing conditions. All mice were housed separated by sex (n = 5 per cage) in polysulfone microisolation cages (30.1 cm × 15.8 cm × 14.1 cm; Super Mouse 750, Lab Products, Seaford, DE), with filter-top lids that provided an additional 0.7 cm above the cage. Filter-top lids were covered entirely with 15-µm filter paper with a surface area of 475.6 cm^2 to the outer rim (Ancare, Bellmore, NY) Prior to autoclaving, all cages were provided with 300 mL of ¼-in. corncob bedding (Bed-o' Cobs, The Andersons, Maumee, OH) and a single 5 cm × 5 cm cotton square (NES3600, Ancare) for environmental enrichment. After autoclaving, cages were maintained on an IVC rack (Lab Products) or placed on a static free-standing wire-shelving rack with 76 cm × 152.4 cm shelves spaced 38 cm apart vertically (3060NS, InterMetro Industries, Wilkes-Barre, PA). Airflow on IVC racks was provided by top-mounted blower and exhaust motors that used HEPA filter air from the room to keep the rack at negative pressure relative to the positive-pressure cages. Air was delivered into cages through a certified airdock at the back base of the cage, and effluent air was captured from cages through a negative pressure canopy and HEPA filtered back into the room. ACH was set by the manufacturer for blower motors and was checked by the service technician prior to initiation of studies to ensure correct function of blower motors and exhaust units. IVC rack ventilation was measured every 48 h at each study-cage location by using a cage monitor unit (Enviro-Gard, Lab Products, Seaford, DE) and verified to be 26.7 ± 0.1 air changes per hour across all used rack locations (n = 238 separate measurements). The number of air changes in the static cage was not measured directly. The number of air changes at the room level was measured as 12.3 under negative pressure.

Mice were fed a standard irradiated diet (7912 Harlan Teklad, Harlan, Madison, WI) and had unrestricted access to chlorine-treated reverse-osmosis–purified water (chlorine, 1.5 ppm; pH 6 to 7) in 100-mL graduated water bottles (Wahmann, Baltimore, MD). Mice were maintained on a 12:12-h light cycle. Water consumption was measured by using the graduations on the bottles, which were read by the same person at the same time of day (1300) for all measurements. Cages without mice were maintained with water bottles and manipulated to account for accidental water (drips) at the same time that water consumption by mice was recorded. Water loss in the absence of mice was subtracted from total water consumption as assumed loss.

Experimental design. Mice were housed for either 7 d under static conditions on a free-standing wire shelving rack or 14 d on an IVC rack. Hydration status of all mice in each cage was assessed at the beginning of the housing period and every 48 h thereafter by measuring weight, TEWL, urine osmolality, and water consumption. Data and samples were collected at the same time each day, to control for possible diurnal variations. Cages were changed after 7 d (static cages) or 14 d (IVC), and mice were switched to the other housing condition.

Temperature and humidity. Intracage temperature and humidity were measured at the level of the bedding by using a hygrometer (model 11-661-16, Traceable Hygrometer Thermometer Dew Point, Fisher Scientific, Waltham, MA) as previously described.[38] This hygrometer was calibrated by the company before experiments began and again before repeating experiments. Cages were modified to allow insertion of the hygrometer probe by drilling a 1/4-in. hole on the midpoint of the front cage surface, 4 cm from the cage bottom. Autoclave tape and a plastic cap were placed over each hole between measurement time points. Temperature and humidity were measured in control cages in the absence of mice. Room humidity was measured by using a traceable hygrometer (Traceable Calibration Control, Friendswood, TX) that is calibrated annually. Room temperatures were measured daily by using a hygro–thermometer (Extech Big Digital, Fotronic, Melrose, MA). All measurements were recorded by the same person at the same time of day for each time point.

Urine osmolality. Urine was collected by free catch or by gentle palpation of the bladder when mice were restrained for TEWL measurement. No urine samples were pooled to obtain a reading. Samples were stored at –20 °C prior to analysis. Osmolality was measured by using a vapor-pressure osmometer (Vapro 5520, Wescor, Logan, UT).[12]

TEWL. Transdermal vapor pressure gradient was measured using a DermaLab Series SkinLab Combo probe (Cortex Technology, Hadsund, Denmark; Figure 1). Measurements are reported in $g/m^2/h$. TEWL measurement requires contact of the probe with hairless skin and takes 30 s or less. In furred mice, hair was removed by using a chemical depilatory on an area just large enough to allow placement of the TEWL probe. Hair removal was performed at least 24 h prior to TEWL measurement[44] and was repeated every 7 to 10 d as necessary.

Statistics. All groups consisted of 2 cages (5 mice per cage) for each strain, sex, and housing condition. To avoid variation, measurements were performed by the same person at the same time each day. Measurements were performed for both housing conditions (IVC and static) at the same time. All experiments were repeated at least once after randomization. Data were analyzed by ANOVA multigroup comparison of means or by Kruskal–Wallis and Mann–Whitney nonparametric tests in InStat version 3.10 for Windows (GraphPad Software, La Jolla, CA). Data are reported as mean ± SEM. The significance level was set at a P value of less than 0.05.

Results

Body weight and water consumption in IVC- and static-housed hairless mice. Body weight did not change significantly over

Figure 1. SCID mouse restrained for placement of the TEWL Probe. The TEWL probe contains paired sensors that measure humidity and temperature and computes 2 separate vapor pressures; the difference between the vapor pressures at the 2 points is used to calculate evaporative water loss from the skin. Restraint time is ≤ 30 s.

time for mice housed in static or IVC conditions (data not shown). Therefore serial weights were averaged for mice within the same sex and strain. Female SCID and SKH mice weighed less than their male counterparts ($P < 0.001$ for all strains). Female SCID ($P = 0.04$), and male SCID ($P < 0.0001$) and male SKH mice ($P \leq 0.0001$) drank less water when housed in IVC as compared with static cages (Table 1). Female SCID mice drank less water than did male mice when housed in static or IVC conditions ($P < 0.05$). When normalized for weight, female SCID mice in both housing conditions and female SKH in IVC drank more water than their male counterparts ($P < 0.05$ for all comparisons).

Urine osmolality in IVC- and static-housed hairless mice. Urine was collected by free catch during manipulation of the mouse for TEWL measurement. IVC- and static-housed SKH mice and IVC-housed SCID mice did not demonstrate any sex-associated difference in urine osmolality. However, because only 2 urine samples were collected from male static-housed SCID mice, statistical analysis for sex-associated differences could not be performed. Data from male and female mice of each strain were therefore pooled for comparison of urine osmolality between static and IVC housing conditions. SCID and SKH mice in static caging had significantly lower urine osmolality (SCID: 3432 ± 200 mOsm/L, $n = 50$; SKH: 3457 ± 179 mOsm/L, $n = 74$) compared with the same strain housed in IVC (SCID: 2071 ± 88 mOsm/L, $n = 17$; SKH: 1959 ± 65 mOsm/L, $n = 50$; $P \leq 0.0001$ for both strains). Consistent with the observed increase in water consumption, SCID and SKH mice housed in static caging had lower urine osmolality compared with mice of the same strain housed in IVC ($P < 0.01$).

Intracage humidity in IVC- and static-housed hairless mice. Humidity levels were measured within the cage starting 24 h after the introduction of mice. Intracage humidity levels were not significantly affected by sex in either SCID or SKH mice (data not shown). Sex-specific data were therefore combined for a given time point, housing condition, and strain. Intracage humidity did not differ significantly between mouse strains housed in IVC (Figure 2 A). Humidity in IVC increased daily at a comparable rate for both SCID and SKH mice and was significantly ($P < 0.05$) elevated from day 7 onward as compared with day 1 values. IVC had significantly ($P = 0.007$) elevated humidity levels starting at day 7 as compared with day one (Figure 2 A). Humidity levels increased more rapidly under static housing conditions, with significant ($P = 0.004$) increases in humidity recorded from day 3 (Figure 2 B). Final intracage humidity levels were higher for static-housed SCID mice (day 7) as compared with IVC (day 13, $P = 0.02$).

TEWL in IVC- and static-housed hairless mice. TEWL levels remained constant over time for each strain and housing condition; data were therefore combined to give a single reading. Consistent with water consumption, SCID mice demonstrated significantly ($P < 0.005$) lower TEWL levels when housed in IVC cages, and female mice had lower TEWL than did male mice (Figure 3). In contrast, SKH mice demonstrated no difference in TEWL regardless of sex or housing condition.

Body weight and water consumption in IVC- and static-housed furred mice. Body weight did not significantly change over time for FVB and C57 mice housed in static or IVC conditions (data not shown). Therefore serial weights were averaged for mice within the same sex and strain. Female FVB and C57 mice weighed significantly less than did male mice of the same strain under both housing conditions ($P < 0.001$ for all strains; Table 2). Consistent with findings in SCID mice, male and female FVB mice drank significantly ($P < 0.05$) less water when housed in IVC as compared with static cages. In contrast, both male and female IVC-housed C57 mice drank significantly ($P < 0.01$) more water as compared with those in static cages. However, only C57 female mice housed in static cages drank significantly less than did C57 male mice. When water intake was normalized for body weight, female C57 mice housed in IVC and static cages drank approximately 20% more water than did male mice under the same housing condition (Table 2).

Urine osmolality in IVC- and static-housed furred mice. IVC- and static-housed FVB mice did not demonstrate any sex-specific difference in urine osmolality (Table 3). Therefore, male and female data for FVB mice were pooled for comparison of urine osmolality between static and IVC housing conditions. Consistent with their increased water consumption, static-housed FVB mice had significantly ($P < 0.01$) lower urine osmolality than did FVB mice in IVC (Table 3), and female C57 mice housed under static conditions had significantly ($P < 0.05$) lower urine osmolality than did male C57 mice. Decreased urine osmolality in female C57 mice may reflect increased water consumption by female mice when normalized for body size. Male and female C57 mice housed in IVC drank more water and had higher urine osmolality than did male and female static-housed mice.

Intracage humidity in IVC- and static-housed furred mice. Humidity levels were measured within the cage starting 24 h after the introduction of mice. Intracage humidity levels were not significantly affected by sex in either FVB or C57 mice (data not shown). Therefore sex-specific data were combined within a given time point, housing condition and strain. Humidity increased daily for IVC and static housing conditions. Intracage humidity was somewhat higher for FVB mice than C57 mice housed under IVC conditions at all experimental time points, although the only significant difference was at 24 h ($P = 0.04$; Figure 4 A). Humidity levels were significantly ($P = 0.03$) elevated in IVC starting at day 7 as compared with day 1. Humidity levels increased more rapidly under static housing conditions than in IVC, with a significant ($P = 0.009$) increase in humidity first recorded at day 3 in static cages (Figure 4 B). There were no differences in humidity levels between IVC- and static-housed FVB and C57 mice (direct comparison by day and overall comparison of day 7 static compared with day 13 IVC).

TEWL in IVC- and static-housed furred mice. Both male and female FVB and C57 mice had significantly ($P < 0.05$) higher

Table 1. Body weight and water consumption of hairless (SCID and SKH) mice in IVC and static housing

	IVC				Static			
	SCID		SKH		SCID		SKH	
	Female	Male	Female	Male	Female	Male	Female	Male
Weight, g	21.1 ± 0.1	26.3 ± 0.1[c]	22.8 ± 0.1	27.6 ± 0.1[c]	20.3 ± 0.2	26.5 ± 0.2[c]	23.3 ± 0.1	26.0 ± 0.3[c]
Water, mL/d	4.0 ± 0.0	4.7 ± 0.0[c]	4. 8 ± 0.1	4.7 ± 0.1	4.4 ± 0.1[a]	5.0 ± 0.1[b,c]	4.7 ± 0.1	5.8 ± 0.2[b,c]
Water, mL/d/25 g	4.8	4.4	5.2	4.3	5.4	4.7	5.1	5.6

Water consumption and body weight were measured every 48 h; weights were averaged for mice within the same sex and strain: $n = 28$ separate measurements per strain and sex for IVC; $n = 16$ separate measurements per strain and sex for static housing. Female SCID and SKH mice weighed significantly less than did their male counterparts ($P < 0.001$ for both strains). SCID female mice drank significantly less water than did male mice when housed in static cages ($P = 0.0003$) or under IVC conditions ($P \leq 0.0001$). Compared with male mice, female SKH mice drank less water when housed in static cages ($P = 0.0009$) but not when housed in IVC ($P = 0.18$). Male and female SCID and male SKH mice drank more water under static housing conditions than in IVC. Data are shown as mean ± SEM.

[a]$P < 0.05$ compared with same strain and sex in IVC

[b]$P < 0.01$ compared with same strain and sex in IVC

[c]$P < 0.05$ sex-associated difference within same strain and housing conditions

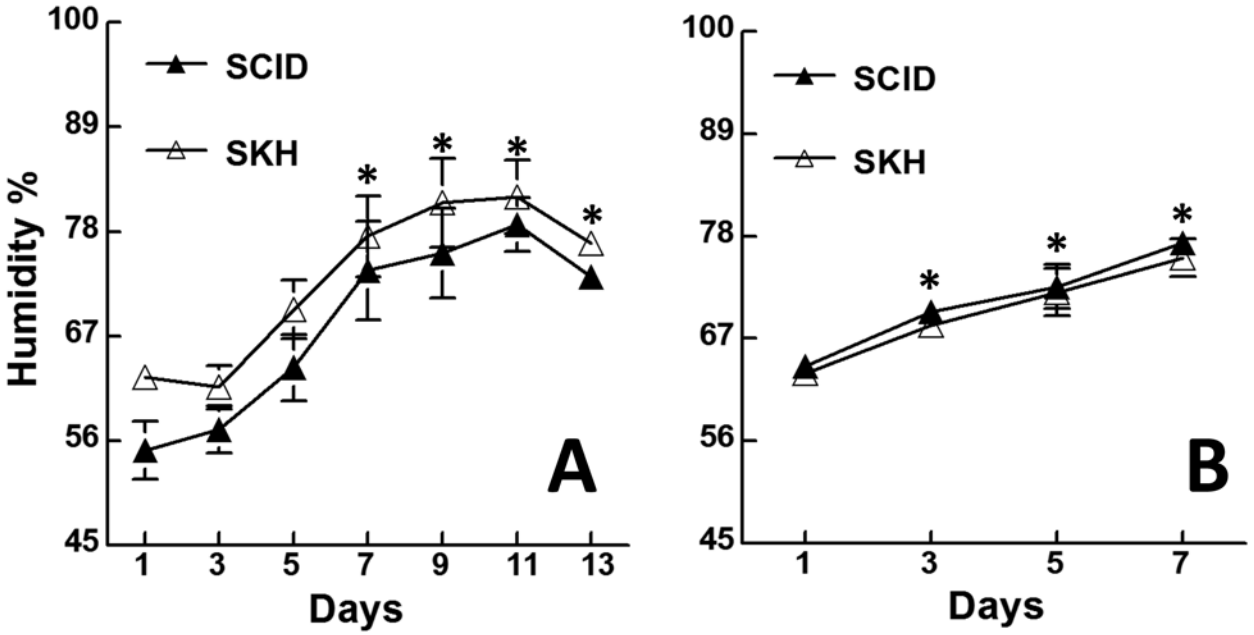

Figure 2. Intracage humidity in IVC- and static-housed hairless (SCID [filled triangles] and SKH [open triangles]) mice. Humidity levels were measured every 48 h starting 24 h after cage change (4 cages per strain and sex). Sex-specific data were combined within a given time point, housing condition, and strain (IVC, $n = 56$ separate measurements per strain; static, $n = 32$ separate measurements per strain). *, Compared with day 1, humidity was significantly increased on day 7 ($P = 0.007$) for IVC and at day 3 ($P = 0.004$) for static housing.

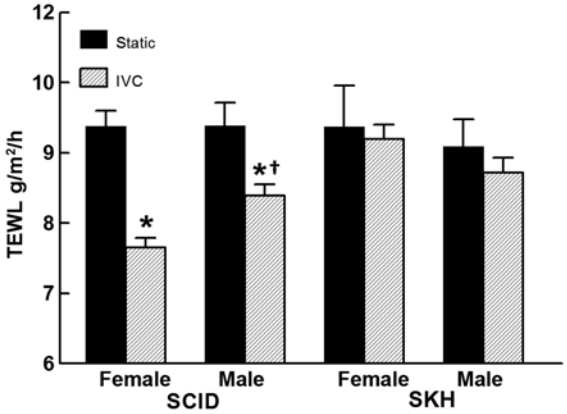

Figure 3. TEWL in hairless (SCID and SKH) mice under IVC and static housing conditions. TEWL was measured every 48 h (5 mice per cage, 4 cages per strain and sex; $n = 40$ per strain and time point for each housing condition). TEWL was significantly decreased in IVC as compared with static housing conditions for both sexes of SCID mice. *, Significant difference between housing conditions for same sex and strain ($P < 0.005$); †, Sex-associated difference ($P < 0.005$) compared with same strain and housing condition.

TEWL when housed under IVC conditions than in static cages (Figure 5). Male C57 mice had significantly ($P < 0.05$) higher TEWL than female mice when housed in either static or IVC conditions. Male FVB mice had higher TEWL than did female mice when housed in IVC. TEWL readings from C57 mice housed in IVC were significantly ($P < 0.05$) higher than those obtained from SCID, SKH, and FVB mice of the same sex under the same housing conditions. Importantly, male C57 mice housed in IVC had significantly higher TEWL than did all other strains and sexes housed either in IVC or static caging. Increased TEWL levels in C57 mice are consistent with increased water consumption and increased urine osmolality; that is, water balance was maintained during increased insensible transepidermal water loss by compensatory increased water intake and decreased sensible water loss in urine.

Micro- and maroenvironmental temperature and humidity. Temperature and humidity were measured within the animal holding room and in empty IVC and static mouse cages with microisolation lids containing bedding and a water bottle. Intracage humidity levels for cages housing mice on study were

Table 2. Body weight and water consumption in furred (FVB and C57) mice in IVC and static housing

	IVC				Static			
	FVB		C57		FVB		C57	
	Female	Male	Female	Male	Female	Male	Female	Male
Weight (g)	24.1 ± 0.2	26.4 ± 0.1[c]	20.1 ± 0.2	25.2 ± 0.1[c]	20.8 ± 0.1	24.7 ± 0.2[c]	17.8 ± 0.1	23.9 ± 0.2[c]
Water mL/d	3.3 ± 0.1	3.7 ± 0.1	4.6 ± 0.2	4.8 ± 0.1	4.1 ± 0.1[a]	4.1 ± 0.1[a]	3.8 ± 0.1[b]	4.2 ± 0.2[b,c]
Water mL/d/25 g	3.4	3.5	5.8	4.8	5.0	4.1	5.3	4.4

Water consumption and body weight were measured every 48 h. Weights were averaged for mice within the same sex and strain; $n = 28$ separate measurements per strain and sex in IVC; $n = 16$ separate measurements per strain and sex in static housing. Female FVB and C57 mice weighed significantly less than did their male counterparts ($P < 0.001$ for both strains). FVB female mice drank significantly less than did FVB male mice in IVC ($P = 0.0069$) but not static cages ($P = 0.78$). Female C57 mice drank significantly less than did male C57 mice in static cages ($P = 0.025$) but not IVC ($P = 0.4$). Male and female C57 mice housed in IVC drank more water under static housing conditions than in IVC. Data are shown as mean ± SEM.

[a]$P < 0.05$ compared with same strain and sex in IVC

[b]$P < 0.01$ compared with same strain and sex in IVC

[c]$P < 0.05$ sex-associated difference under same strain and housing conditions

Table 3. Urine osmolality (mOsm/L) in IVC- and static-housed furred (FVB and C57) mice

	IVC				Static			
	FVB		C57		FVB		C57	
	Female	Male	Female	Male	Female	Male	Female	Male
Sex-specific	3490 ± 237	3093 ± 137	1980 ± 87	2403 ± 318	2292 ± 58	2224 ± 60	1328 ± 65[b]	1683 ± 85[a,c]
n	50	56	44	38	28	33	56	36
Overall	3280 ± 133		NA		2255 ± 41[b]		NA	
n	106				61			

NA, data not combined due to sex-associated differences

Sex-specific differences were not identified for FVB mice, so sex-specific data were pooled for the strain. Static-housed FVB mice had significantly lower urine osmolality than did those housed in IVC ($P \leq 0.0001$). C57 mice had significant differences in urine osmolality in static ($P = 0.001$) but not IVC housing. Data are shown as mean ± SEM.

[a]$P < 0.05$ compared with same strain and sex in IVC

[b]$P < 0.01$ compared with same strain and sex IVC

[c]$P < 0.05$ sex-associated difference under same strain and housing conditions

averaged across all time points for comparison with those of the room and control cages without mice. Both IVC and static cages housing mice had significantly ($P < 0.01$) higher intracage temperature and humidity levels as compared with the control cage in the absence of mice (Table 4). In addition, IVC systems had higher temperatures and lower humidity levels than did static cages. The use of the microisolation lid appeared to increase temperatures within the cage by approximately 2 °F relative to the holding room for both IVC and static conditions; the presence of microisolation lids on empty cages increased intracage humidity. The addition of mice to cages increased temperatures by 7 to 12 °F (4 to 7 °C) for IVC and 7 to 10 °F (4 to 6 °C) for static cages as compared with that of the animal holding room. The addition of mice to cages increased humidity from 27% within the mouse room to 68% to 79% for IVC and 70% to 73% under static conditions.

Discussion

Water metabolism is a simple balance between water intake and loss. In mice, the primary types of water loss include sensible loss via urine and insensible loss through the respiratory tract and skin. Mice conserve moisture and respond to increased temperatures by using evaporative heat loss for cooling rather than by increasing respiration.[18] The influence of environmental and genetic factors on food and water consumption by mice is well documented.[2,18] Body weight and urine osmolality are 2 of the most widely used indices for hydration status.[1,23] Serial measurement of body weight alone has been used successfully as an index for hydration status in mice,[12] with water intake in normally hydrated animals being proportionate to body weight.[18] The purpose of the current study was to determine the influence of ventilated and static housing conditions on sensible and insensible water loss in common strains of hairless (SCID and SKH) and furred (FVB and C57) mice.

Water consumption occurs as part of the normal circadian rhythm for mice with the highest drinking taking place at night.[13,33,35,36] Water is consumed in a synchronous pattern with food; however, water consumption is not driven by food consumption but is a separately controlled behavior. Water consumption is thought to be regulated genetically by a common biologic clock in all mice, resulting in similar patterns of drinking.[36] A separate mechanism controls absolute amounts of water consumed, resulting in the differences seen between strains.[36] To minimize variation in the current study, the same mice were used for water-consumption measurements under both static and IVC housing conditions, and all measurements were performed at the same time of day. Body weight as an indication of hydration status did not change over the course of this study, regardless of housing condition, and female mice were consistently smaller than male mice of the same age and strain. However, when normalized for body weight, female SCID and C57 mice drank more water under both housing conditions. We hypothesized that the use of IVC would result in lower intracage humidity and higher water consumption by all strains of mice; however, hairless SCID and male SKH as well as white-furred FVB mice drank more water when housed under

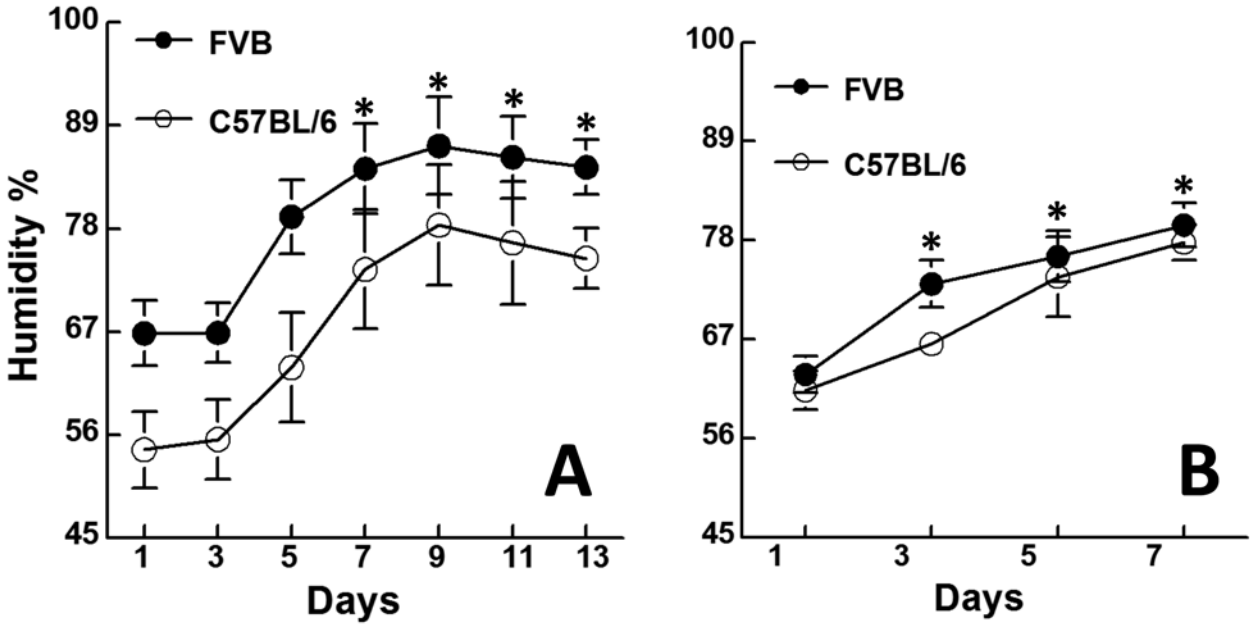

Figure 4. Intracage humidity in IVC- and static-housed furred (FVB [solid circles] and C57 [open circles]) mice. Humidity levels were measured every 48 h starting 24 h after cage change: 4 cages per strain and sex. Sex-specific data were combined within a given time point, housing condition, and strain (IVC, n = 56 separate measurements per strain; static, n = 32 separate measurements per strain). *, Compared with day 1, humidity was significantly increased at day 7 (P = 0.03) for IVC, and at day 3 (P = 0.009) for static housing.

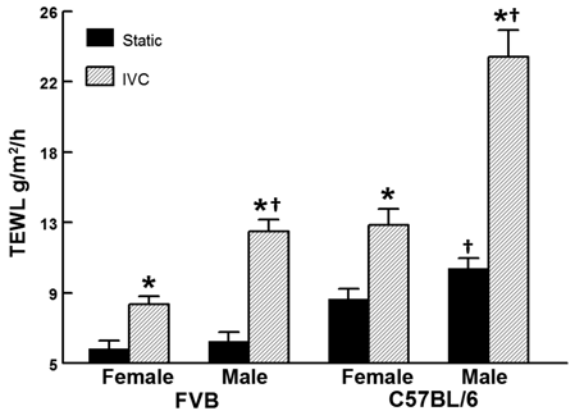

Figure 5. TEWL in furred (FVB and C57) mice under IVC and static housing conditions. TEWL was measured every 48 h: 5 mice per cage, 4 cages per strain and sex; n = 40 per strain and time point for each housing condition. TEWL was significantly increased in IVC as compared with static housing conditions for both strains and sexes. *, Significant difference between sexes and strains under static housing. †, Sex-associated difference in same strain under IVC housing conditions; p = 0.046 sex-associated difference in same strain under static housing condition.

static conditions. Water consumption increases in C57 mice fed a high salt diet (4%);[42] however, all mice were fed a standard rodent chow with a salt content of 0.8% in the current study, such that dietary salt was not considered as a factor for this study.

Urine output and osmolality are indicators of sensible water loss. Urine osmolality reflects the urine-concentrating mechanism fundamental for the regulation of water excretion. Decreased water intake results in the production of more concentrated urine that is hypertonic to blood plasma.[40] Sex-associated differences in urine concentrating capabilities have been reported for humans[17,34] and rats.[50] However, a systematic examination of the influence of sex on urine-concentrating ability in mice has not been performed. Overall, urine osmolality values measured in the current study are consistent with previous reports of urine osmolality values in mice.[43] No sex-specific differences were identified for SCID, SKH, and FVB mice under either housing condition. Importantly, however, urine osmolality was higher in male C57 mice than female C57 mice housed in static caging. Because diurnal regulation of urine production has been reported to occur in men and women,[16] urine was collected at the same time each day in the current study. However, it remains possible that interstrain differences in the diurnal regulation of urine production might account for the inability to detect any sex-associated differences in osmolality except for those in C57 mice. Regardless of any sex-specific difference, these data represent the first report of urine osmolality values for SCID, SKH, FVB, and C57 mice.

Nearly 80% of insensible water loss in mice occurs through the respiratory tract.[18] Water loss through the respiratory tract occurs at the level of the alveoli and is dependent on several physiologic parameters, including body size and temperature and the alveolar ventilation rate (respiratory rate). Because the alveolar partial pressure of water vapor (P_AH_2O) is constant, water loss through the respiratory tract cannot be easily regulated and is considered to occur at a constant rate.[14,18] Evaporative heat loss is primarily accomplished by TEWL. Because mice do not increase respiration in response to increased temperatures (that is, pant), increased water evaporation through skin is an important mechanism by which cooling (heat loss) can occur. TEWL is a physiologically significant source of insensible water loss (20%) that is modulated by environmental parameters. Therefore, differences in rates of evaporative heat loss through skin between strains of mice will significantly affect total insensible water loss. The TEWL probe calculates evaporative water loss from the skin by using a set of vapor-pressure probes. TEWL has primarily been used in mice to understand the barrier function of the epidermis under experimentally disturbed or diseased conditions.[15,30,41,49]

The current studies used TEWL under routine housing and husbandry conditions in an attempt to better understand water

Table 4. Micro- and macroenvironmental temperature (°F) and humidity (%)

	IVC						Static				
	Room	Control	SCID	SKH	FVB	C57	Control	SCID	SKH	FVB	C57
Temperature	69.3 ± 0.4	71.6 ± 0.5	78.3 ± 0.4[a]	80.6 ± 0.3[a]	81.4 ± 0.3[a]	76.6 ± 0.3[a]	71.6 ± 0.3	76.6 ± 1.2[a,b]	78.0 ± 0.6[a,b]	79.4 ± 0.6[a,b]	76.2 ± 0.5[a]
Humidity	27.3 ± 2.7	45.8 ± 0.5	68.2 ± 1.7[a]	73.0 ± 1.6[a]	79.2 ± 1.8[a]	68.0 ± 2.3[a]	34.8 ± 4.2	70.9 ± 1.2[a]	69.8 ± 1.2[a]	73.0 ± 1.6[a,b]	69.9 ± 1.7[a]

Intracage temperature and humidity levels were measured every 48 h. Humidity within the animal holding room was measured every 2 wk. For comparison with the animal holding room, all intracage temperature and humidity values were averaged for a single strain (IVC, $n = 56$; static, $n = 32$). Both IVC and static mouse cages had significantly ($P < 0.05$) higher temperature and humidity levels as compared with the room levels. IVC systems had higher temperature levels as compared with static cages ($P < 0.05$). Cages with mice had a temperature increase of 7° to 12 °F for IVC and 7° to 10 °F for static cages as compared with the animal holding room. Cages with mice had humidity levels that were >150% increased for both static and IVC as compared with the room alone. Animal holding room, $n = 9$; control cages without mice $n = 14$. Data are shown as mean ± SEM.

[a]$P < 0.01$ compared with control under the same conditions

[b]$P < 0.01$ compared with same strain in IVC

regulation in these strains. Humidity alters insensible water loss and TEWL in furred mice[18] and hairless mice.[9,41] Environmental humidity has a direct effect on skin cell turnover, skin function, and TEWL.[9] Humidity levels for TEWL measurement have been defined as high (>80%), normal (40% to 70%), and low (<10%);[9,11,41] however, a dose–response relationship in the influence of humidity on TEWL has not been reported. Likewise, water consumption has been inversely correlated with environmental humidity.[32] Higher intracage humidity has been associated with decreased water consumption by mice housed in both IVC (compared with open-top cages)[52] and static (closed top) microisolation cages.[32] Humidity was measured at the room level and within the cage as a function of TEWL levels; that is, as humidity decreases, TEWL levels or water consumption are expected to increase to maintain physiologic water balance. In the current study, TEWL levels remained constant over time, indicating that although intracage humidity significantly increased over time until cage change, the intracage starting humidity levels (>50%) were already at the maximal saturation point for affecting water loss by TEWL.

Hairless SCID mice—but not SKH mice—demonstrated sex-specific differences in TEWL under different housing conditions. Hairless SKH mice have been shown to adapt to environmental changes (humidity levels) by altering the structure and function of the epidermis to maintain water balance,[41] and this response may account for the constant rate of TEWL regardless of housing condition. Likewise, distinctive structural differences between the epidermis of nude and hairless mice[6] may influence TEWL. A perceived limitation of this study is that TEWL measurements only represent the rate at which water is lost (limited by size of the TEWL probe per specified time). Calculation of total water loss requires use of the total body surface area, a number that is currently unavailable for all the strains we used in this study.[8] Although comparing the relative rates of water loss is useful for understanding water metabolism, caution should be applied when comparing this information with absolute water consumption over time with special consideration of the size of the animal.

Interestingly, and despite the presence of fur, both FVB and C57 mice had higher TEWL levels than hairless SCID and SKH mice. IVC housing resulted in a higher TEWL for FVB and C57 mice. The ability of furred mice to alter the epidermis and TEWL in response to differences in environmental humidity has not been reported previously. Moreover, hair was removed from FVB and C57 mice by using a chemical depilatory every 7 to 10 d. Depilation of furred mice for surgical or experimental procedures is a standard practice in biomedical research. The use of depilatory creams for hair removal is effective, nontoxic, and significantly reduces skin-surface bacteria.[21] Although the removal of hair is a standard for the use of TEWL in furred mice,[44] this process might disrupt or damage the epidermal barrier. These data imply that furred mice that have been treated to remove hair (depilatory cream or shaving) for surgical or experimental procedures may require unrestricted access to water to maintain hydration status. The high TEWL levels specifically in C57 mice may reflect accepted inflammatory skin changes associated with this strain[22,47] that may be exacerbated by depilation. Regardless, the increased TEWL in IVC-housed as compared with static-housed C57 mice parallels the greater water consumption and urine osmolality necessary to maintain a normal hydration status for this strain under this condition. Importantly, male C57 mice housed in IVC had significantly higher TEWL than did all other strains and sexes housed in either IVC or static caging. Given the widespread use of C57 mice and genetically modified C57 congenic mice in biomedical research, this difference may be of considerable importance, particularly in studies of renal function.

Currently, whether the conditions for mice recommended by the *Guide for the Care and Use of Laboratory Animals*[19] are appropriate to maintain mice in their thermoneutral zone is unclear.[3,10,24,26,27,45] Humidity was measured within the animal holding room as an indication of starting levels for intracage humidity. Intracage humidity for IVC and static cages has been reported to have an increased difference of 5% to 10% as compared with the animal holding room.[32,37-39] We hypothesized that IVC caging would alter water metabolism by reducing intracage humidity and temperature levels as compared with those in static cages. In contradiction to our expectations, average intracage temperature and humidity levels were much higher than those of the animal holding room, regardless of housing condition, mouse sex, or strain. It should be noted that, for the purposes of this study, statistics were performed for comparison of temperature and humidity between control cages in the absence of mice and cages housing mice. Average humidity was lower in IVC than static cages; however, the humidity range for IVC remained well within recommendations from the *Guide*[19] and was more than 150% higher than the humidity recorded within the animal holding room. Likewise temperatures within mouse cages ranged from approximately 77 to 81 °F for IVC and 77 to 79 °F for static conditions as compared with 72 °F in control cages or 69 °F within the animal room. Again, these temperatures did not differ from *Guide* standards.[19] Previous studies have reported that temperatures are about 2 °F higher in IVC than the animal holding room; however, those studies used IVC with 60 to 98 complete air-changes hourly,[11] whereas in the current studies a rate of 26.7 ± 0.1 ($n = 238$ separate

measurements) air-changes hourly was recorded. Although the location at which intracage environmental parameters are gauged can affect the recorded measurement,[32] it should be noted that in the present study, intracage temperature and humidity were determined at the level of the bedding in all cages to ensure consistency of results. Finally, cages maintained with a full water bottle but without mice had an increase in temperature of approximately 3 °F and an increase in humidity of 7% to 19% as compared with measures in the animal holding room. It is unclear why cages maintained in the absence of mice were warmer than the animal holding room, but this situation may reflect differences in recording equipment or air that was warmed by movement through the automated rack system. The presence of increased humidity in the absence of mice was thought to be caused by evaporation or water leakage from the water bottle during manipulation of the empty cage.

These data are the first that document the rate of insensible water loss through the skin by using TEWL and urine osmolality values for SCID, SKH, FVB, and C57 mice. The ability of SKH and SCID mice to regulate epidermal function to retain water and maintain water balance contradicts the assumption that hairless mice housed in IVC might be prone to dehydration.[28] However, increased TEWL in furred mice housed in IVC may indicate that the epidermis of these strains may not compensate for depilation, thereby increasing the risk for dehydration to mice that may already be compromised due to experimental manipulation. The C57 strain is one of the primary mouse strains used in biomedical research.[20] Although none of the C57 mice in these studies exhibited signs of skin disease, this strain has been shown to have an increased incidence of dermatitis.[22] Altered vitamin A metabolism, excessive grooming, and the robust T-helper immune response of this strain have all been proposed to contribute to subacute changes in the dermis of these mice.[22,47,51] Significantly higher TEWL levels in C57 mice as compared with the other strains may reflect immune-mediated or metabolic changes in the skin that ultimately affect water metabolism. The increased TEWL in C57 mice housed in IVC is a strain-specific difference that should be considered in studies involving renal function or water restriction. Finally, there is increasing discussion regarding the concept of 'cold stress' in mice housed under conditions outlined in the *Guide*.[19] The data from our current studies indicate that mice were significantly warmer than the animal holding room, regardless of housing condition and the presence of fur.

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