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Effects of Daytime Blue-Enriched LED Light on Physiologic Parameters of Three Common Mouse Strains Maintained on an IVC System

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Light has been a crucial part of everyday life since the beginning of time. Most recently, light-emitting diode (LED) light enriched in the blue-appearing portion of the visible spectrum (465 to 485 nm), which is more efficient in energy use, is becoming the normal lighting technology in facilities around the world. Previous reports revealed that blue-enriched LED light at day (bLAD) enhances animal health and wellbeing as compared with cool white fluorescent (CWF) lighting. We hypothesized that bLAD, compared with CWF light, has a positive influence on basic physiologic indices such as food consumption, water consumption, weight gain, nesting behavior, complete blood count, and blood chemistry profile. To test this, we allocated 360 mice into equal-sized groups by sex, strain (C3H/HeNCrl, C57BL/6NCrl, BALB/cAnNCrl), lighting conditions, and 6 blood collection time points (n = 5 mice/sex/strain/lighting condition/time point). Food consumption, water consumption, body weight, nest location, and nest type were recorded every 3 d. At the end of the study, all mice were anesthetized over a period of 1 wk and blood was collected via cardiocentesis at 6 different time points. Overall, male C3H/HeNCrl consumed more food under bLAD conditions as compared with CWF conditions; male C3H/HeNCrl had lower cholesterol levels under bLAD conditions than under CWF conditions; female BALB/cAnNCrl mice had higher serum total protein under bLAD conditions than under CWF conditions; female C57BL/6NCrl mice had higher phosphorus levels under bLAD conditions than under CWF conditions, and female C3H/HeNCrl mice had a higher neutrophil count under bLAD conditions as compared with CWF conditions. Although sex and strain differences were found in various physiologic parameters under bLAD as compared with CWF lighting conditions, the differences were minimal. Thus, this study suggests that for these strains of mice, bLAD and CWF are largely equivalent with regard to indices of health and wellbeing, although some differences could affect research outcomes.

Abbreviations: bLAD,blue-enriched light-at-day; CWF, cool white fluorescent; LED, light-emitting diode; SCN, suprachiasmatic nucleus

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Light plays a pivotal role in many cellular processes of eukaryotes and prokaryotes alike. For thousands of years, the only light sources available were sunlight, moonlight, and fire. During the Industrial Revolution, light exposure began to change with the introduction of artificial electric lighting technologies. For a little over 130 y, indoor and outdoor light fixtures have used broad-spectrum incandescent carbon arc and gas discharge lighting systems, such as cool white fluorescent and neon lights. More recently, the emergence of light-emitting diode (LED) technology has begun to replace conventional broad spectrum cool white-fluorescent lighting systems. While some research has been done, we still do not know the full health implications of these lighting systems on laboratory animals and humans.

Light stimulates various rods and cones in the retina via the primary optic tract, giving a multitude of animals a sense of vision.[3,14] Mice have a rod-dominant retina, appropriate for their nocturnal nature. However, mice have 2 types of cones that respond to light, S-opsin expression only and S-opsin plus M-opsin expression. S-opsin expression only cones, which account for only about 5% of cones in the mouse retina, have peak sensitivity in the 360 nm range, while S-opsin plus M-opsin expressing cones have peak sensitivity range in both the 360 nm and 508 nm range.[34] These differences warrant investigation of the effects of LED lighting in the 465 to 485 nm range, as compared with the broader spectrum cool white fluorescent (CWF) lighting, on laboratory animals such as mice.

In addition, the nonimage-forming visual system of the retinohypothalamic tract transmits photic signals from the retina and a small subset of ganglion cells called the intrinsically photosensitive retinal ganglion cells to the anteriobasal portion of the hypothalamus called the suprachiasmatic nucleus (SCN) or master biologic clock. The SCN plays a pivotal role in circadian rhythms and physiologic and hormonal regulation in any organism.[26,32] The SCN has been evolutionarily conserved from lower taxa animals to higher ones.[28] The SCN regulates the daily dark phase pineal production of melatonin, resulting in high dark phase and low light phase levels.[7,9,10,30] Daily melatonin contributes to the temporal coordination of normal mammalian behavioral and physiologic functions. Previous research has demonstrated that C3H/HeNCrl inbred mice and nude rats have a higher dark phase melatonin peak and prolonged elevations in melatonin levels under blue-enriched light-emitting

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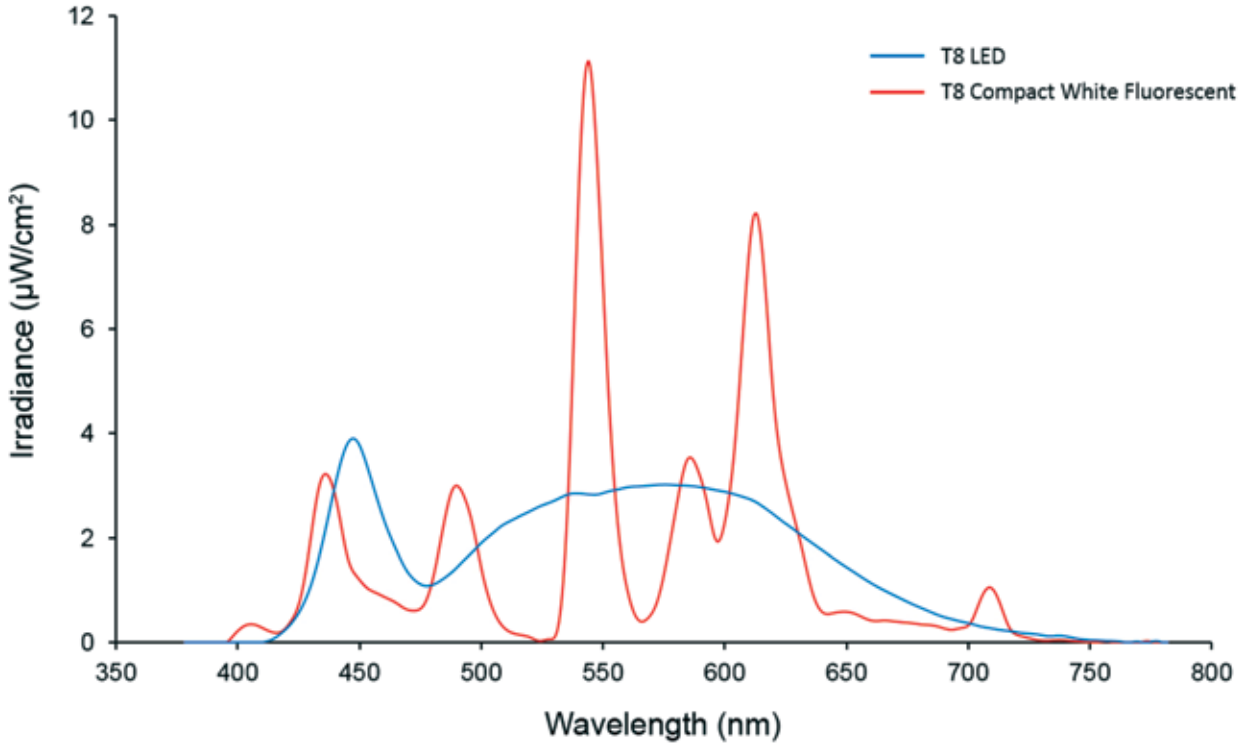

Figure 1. Normalized spectral power distributions of the blue-enriched LED (blue) and cool white fluorescent (red) light transmitted through a standard polycarbonate, translucent laboratory mouse cage.

Table 1. Light intensity

	CWF (n = 108)			bLAD (n = 108)			
Variable	Mean	Std Dev	Std error	Mean	Std Dev	Std error	P
µW/cm^2	25.2	5.24	0.5	24.1	6.47	0.62	0.1799
Melanopic lux	180.6	37.6	3.6	172.8	46.4	10.9	0.1799

T-Test results of light intensity in bLAD and CWF. Degress of freedom is equal to 214 (108 – 1) + (108 – 1).

diode (LED) during the light phase (bLAD).[7,9,10] This effect was associated with positive metabolic and physiologic effects such as decreased tumor growth and lower levels of arterial serum total fatty acid, corticosterone, insulin, and leptin.[7,9,10]

Although more research is appearing on light quality and quantity effects on animal physiology, much is still unknown regarding the effects of light on basic physiology. Because light affects hormones such as melatonin, it also has the potential to effect electrolyte homeostasis, including calcium and phosphorus. Therefore, we sought to study strains of mice that produce circadian dark phase melatonin (C3H/HeNCrl) as compared with mice that do not (BALB/cAnNCrl and C57BL/6NCrl).[29] In addition, light plays a role in regulating cell populations, behavior, and stress. For example, exposure of rats to light at night increases corticosterone levels at 8 h before the normal circadian peaks of corticosterone.[8] One report states that the quality of light during the day has a positive effect on human workers dealing with "social jetlag."[15] One way to assess basic physiologic indices in animals is through a complete analysis of blood count and blood chemistry. Therefore, this study compared the effects of bLAD and CWF light in mice by performing a complete analysis of blood count and blood chemistry in addition to basic physiologic parameters (weight, water intake, feed intake) and nesting behavior. We hypothesized that mice exposed to bLAD, particularly C3H/HeNCrL mice, will have positive physiologic and behavioral indices, such as lower body weight, lower cholesterol levels, lower indices of stress, more completely built nests, etc., than do their CWF counterparts.

Materials and Methods

Animals, housing conditions, and diet. A total of 360 4-wk old mice, 60 male and 60 female BALB/cAnNCrl inbred (Charles River Lab strain code 028), 60 male and 60 female C57BL/6NCrl inbred (Charles River Lab strain code 027), 60 male and 60 female C3H/HeNCrl inbred (Charles River Lab strain code 025) *Mus musculus*; were purchased from Charles River (Wilmington, MA). Hereafter, these strains will be referred to as BALB/c for BALB/cAnNCrl, B6 for C57BL/6NCrl, and C3H for C3H/HeNCrl. Animals were maintained in an AAALAC-accredited facility in accordance with *The Guide for the Care and Use of Laboratory Animals*.[21] All procedures for animal use were approved by the Tulane University IACUC. Upon arrival, mice were randomly and equally distributed by sex, strain, and controlled lighting group (either CWF or bLAD). Mice were grouped 5 to a cage by sex and strain.

Mice were housed in standard translucent, clear, ventilated laboratory cages (19.05 × 27.94 × 12.7 cm; wall thickness 0.25 cm; polycarbonate, single-sided 7115 Series; Allentown, NJ) containing hardwood maple bedding (P.J. Murphy, Montville, NJ). Bedding changes were performed every 3 d. Environmental enrichment was provided as a 4-gram white shredded paper nesting material (Bed-r' nest, The Andersons, Maumee, OH).

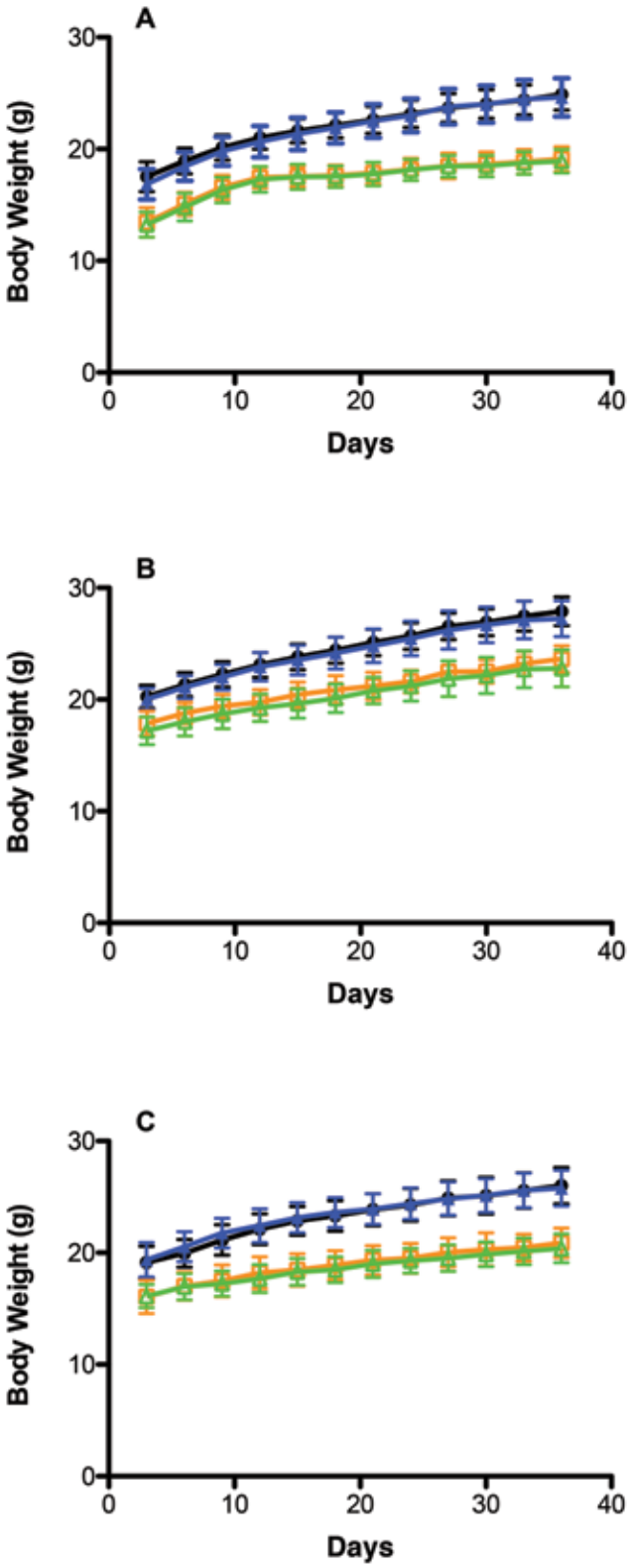

Figure 2. Body weight changes in grams (mean ± 1 SD; n = 30 per group) of male and female BALB/c (A), C3H (B), and B6 (C) mice maintained fed normal chow ad libitum and maintained on either control CWF (male, solid black circles; female, open amber squares) or experimental bLAD (male, solid blue triangles; female, open green triangles) lighting conditions.

The animals were maintained in environmentally controlled rooms (25 °C; humidity 20% to 55%) with diurnal lighting (12:12-h light:dark cycle; light on, 0600). To ensure that all mice remained infection-free from both bacterial and viral agents; serum, fecal, and fur swab samples were tested at completion of the study using multiplex fluorescent immunoassay 2 for serum testing and PCR for fecal and fur testing on sentinel animals (2 female, CRL:CD1(ICR), Charles River Lab strain code 022, mice 5 to 6 wk of age per lighting condition) exposed to dirty bedding (IDEXX Research Animal Diagnostic Laboratory, Columbia, MO). Sentinels were found to be negative for ectromelia virus, EDIM, lymphocyctic choriomeningitis virus, *Mycoplasma pulmonis*, mouse adenovirus type 1, mouse adenovirus type 2, mouse hepatitis virus, mouse norovirus, mouse parvovirus, mouse minute virus, mouse polyoma virus, pneumonia virus of mice, reovirus 3, Theiler murine encephalomyelitis virus, Sendai virus, *Aspiculuris tetraphera, Syphacia muris, Syphacia obvelata, Myocoptes, Radfordia,* and *Myobia*.

Throughout the experiment, food intake, water intake, and body weights were measured every 3 d. Animals received free access to diet (number 5053 Irradiated Laboratory Rodent Diet, Purina, Richmond, IN) and acidified tap water (pH approximately 2.8). Feed and water intake are difficult measurements to assess in mice and the literature has presented multiple ways of collecting such data. A common way to measure feed and water intake is to premeasure all water and feed and to subtract the remaining feed after a period of time and divide by 100 g to determine how much food has been consumed.[6] However, this approach is based on each mouse (if there are 5 mice per cage) weighing 20 g. This assumption is not valid for mice that are rapidly growing animals as at the beginning of our experiment. In our study, some mice weighed less than 10 g at the start of the experiment, and by the end of the experiment some mice weighed greater than 30 g. Therefore, for feed and water intake calculations, the feed, water and body weights were measured at the beginning and at the end of a 3 d measurement period were used to calculate feed and water intake over 3 d periods using 2 formulas. Using days 9 and 12 as examples, the 2 formulas were: [[[feed weight on day 12—feed weight on day 9]/ 3 d]/ weights of mice on day 9] and [[[feed weight on day 12—feed weight on day 9]/ 3 d]/weights of mice on day 12]. Those 2 values were then averaged to get the closest approximation to grams of feed consumed per kilogram of mice per day over a 3 d period. Water was calculated in a similar fashion. Spillage of feed and water was not accounted for, as these are thought to be minimal (less than 0.1g/day feed and less than 0.1mL/day water) relative to food and water consumed.[1,2]

Caging, lighting regimens, and spectral transmittance measurements. The CWF control animal room was lighted with a series of 2 overhead luminaires containing 4 standard soft, cool-white (2700 lm; 4100 correlated-color temperature) fluorescent lamps per ballast (F32T*TL841, model 272484, Alto II Collection, 32 W, 48 in. Series 800, Philips, Somerset, NJ). The experimental LED animal room was lighted with a series of 2 overhead luminaries containing 4 LED lamps, high in emission of blue-appearing portion of the visible spectrum (465 to 485 nm, 2650 lm: 5000 color-correlated temperature) lamps per ballast (12T8/AMB/48 [model 9290011242], T8 12 W, 48 in., Philips, Somerset, NJ). Animal rooms had no light contamination during the dark phase. Lighting, lighting regimens, and spectral transmittance measurements have been described in detail previously.[7-10] Normal light-phase lighting intensity was measured weekly at 1 m above the floor in the center of the room, to the left, and to the right of the IVC rack. Light measurements were made at 6 locations within every cage (rear corners, middle sides, and front corners) prior to introducing the animals and at completion of the study. Irradiance measures were recorded using a

Table 2. Body weight, light, and light:day interactions

		CWF		bLAD		F p	
Strain	Sex	Mean	Std Dev	Mean	Std Dev	Light	Day:Light
BALB/c	Male	22.0	2.5	21.8	2.8	4.66 **0.0349***	0.41 0.9522
BALB/c	Female	17.4	1.9	17.3	1.9	3.02 0.0876	0.10 0.9999
C3H	Male	24.6	2.6	24.3	2.7	8.99 **0.0040***	0.15 0.9993
C3H	Female	21.0	2.1	20.4	2.3	41.27 **<0.0001***	0.27 0.9906
C57BL/6	Male	23.2	2.6	23.4	2.4	3.08 0.0848	0.45 0.9324
C57BL/6	Female	18.9	2.0	18.6	1.7	10.62 **0.0019***	0.32 0.9815

This table displays means and standard deviations averaged over the entire time period. Significant differences marked by (*) denote light effects on weight over the entire period. Day*Light interactions were not statistically significant among all strain and sex combinations.

radiometer-photometer (model no. IL-1400A, International Light Technologies, Peabody, MA) with a silicon diode detector head (model no. SEL033), which included a wide-angle input optic (W6849) and filter (F23104) that provided a flat response across the visible spectrum. Illuminance measures used a silicon diode detector head (model no. SEL033), which included a wide-angle input optic (W10069) and filter (Y23104) to provide a photopic illuminance response. The meter and associated optics were calibrated annually at International Light Technologies.

At approximately 0800 every day, all cages were rotated one position to the right on the IVC rack in the same horizontal plane. If no more slots were available for cages on the same horizontal plane to the right, the cage would then be moved to the next row furthest slot to the left. The daily cage shift ensured uniformity of intensity of ocular light exposure by the animals and accounted for subtle differences due to position on the rack. Measures of melanopic lux, appropriate for light phase vision assessment, are reported along with radiometric values of irradiance (µW/cm2), which are appropriate for quantifying light stimulus that regulate circadian, neuroendocrine, or neurobehavioral physiology in animals and humans. Melanopic lux was calculated using the provided excel file found at http://lucasgroup.lab.manchester.ac.uk/research/measuringmelanopicilluminance/.[33]

Spectral Power Measurements. The spectral characteristics of each light source were taken separately by using a handheld spectroradiometer (ASD, FieldSpec, ASD, Boulder, CO) with a cosine receptor attachment. A measure of the concentration (as a function of wavelength) of the radiometric quantity (that is, irradiance compared with wavelength), or spectral power distribution, was recorded as the meter was pointed upward and directly at the lighting source at a distance of 30 cm with an exposure time of 1 second. This procedure was performed once prior to initiation of the study.

Blood collection. After 36 d of exposure to the lighting regimens, terminal blood collection was performed by cardiocentesis over a 1 wk period. Sampling times were 0400, 0800, 1200, 1600, 2000, 2400. To manage the number of mice being sampled, blood collection began 2 h before and ended 2 h after the aforementioned times. Prior to blood collection, mice were anesthetized with a ketamine (Zetamine, VetOne, Boise, ID)/xylazine (XylaMed, VetOne, Boise, ID) mixture, 80/8 mg/kg respectively, IP via 100-U insulin syringe (Becton Dickinson, Franklin Lakes, NJ). Mice were then placed in dorsal recumbency. A 5/8 in 25 G needle (Covidien, Minneapolis, MN) and 1 mL syringe (Covidien) was introduced at a 30° to 45° angle below the xiphoid process. Blood was drawn until exsanguination was achieved (approximately 0.8 to 1 mL), which was followed by cervical dislocation. Exsanguination took less than 15 seconds per cardiocentesis procedure. Appropriate aliquots of blood were distributed into micro serum separator tubes and micro EDTA tubes provided by IDEXX Bioanalytics (West Sacramento, CA). EDTA samples were refrigerated at 4 °C for a period less than 1 wk before analysis. Serum samples were centrifuged (Centrifuge 5415C, Eppendorf, Hamburg, Germany) for 15 min at 1305 x *g* and then refrigerated at 4 °C or frozen at -20 °C in a frost-free freezer. Both methods of storage were recommended by the diagnostic lab; variation between these 2 storage methods is minimal.[22] All samples were sent within 7 days after collection to IDEXX Analytic (West Sacramento, CA) for complete blood count and blood chemistry analysis.

Nesting Behavior. Nesting behavior data was collected every 3 d when cages were changed. Nest type was qualitatively assessed based on descriptions reported in the literature.[16] as flat, bowl (cup), or dome shaped nests In addition, the location of the nest within the cage was recorded as either front left, front middle, front right, center left, center middle, center right, rear left, rear middle, or rear right.

Statistics. Statistical analysis was performed using SAS software (SAS version 9.4, Cary, NC). For analysis of food intake, water intake, and behavior, each cage was treated as a unit (n = 6 cages each for sex, strain, and lighting condition). For analysis of body weights, n = 30 mice for each sex, strain, and lighting condition. Light intensity was compared using a Student t test. Body weights, water intake, and feed intake were analyzed using a repeated measures ANOVA implemented through a mixed model regression framework with days and light condition as factors; analyses were conducted separately for each strain/sex combination. If the day*light interaction was significant, the effect of light at each time point was assessed using t test on least square means. We did not adjust for multiple comparisons after a significant finding because these were preplanned analyses. Blood chemistry and complete blood count were analyzed using a Wilcoxon–Rank Sum Test. Nesting behavior could not be analyzed statistically due to low statistical power; therefore, percentage values were calculated for nesting type and location. Unless otherwise noted, all values are presented as mean ± 1 SD (SD). Differences were considered to be statistically significant at a 2-tailed P value of less than 0.05. Figures were constructed using Prism 8 (GraphPad Software, La Jolla, CA).

Results

Irradiance measurements peaked in the 460 to 480 nm range for blue-enriched LED light (Figure 1). Irradiance measurements peaked at various wavelengths between the 400 to 725 nm range under CWF light conditions. No significant difference was detected between bLAD and CWF light when light intensity was compared (Table 1) ($P > 0.05$). The mean within-cage light intensity under CWF conditions was 25.2 ± 5.24 µW/cm^2 (180.6 ± 37.6 melanopic lux). The mean within-cage light intensity under bLAD conditions was 24.1 ± 6.47 µW/cm^2 (172.8 ± 46.4 melanopic lux).

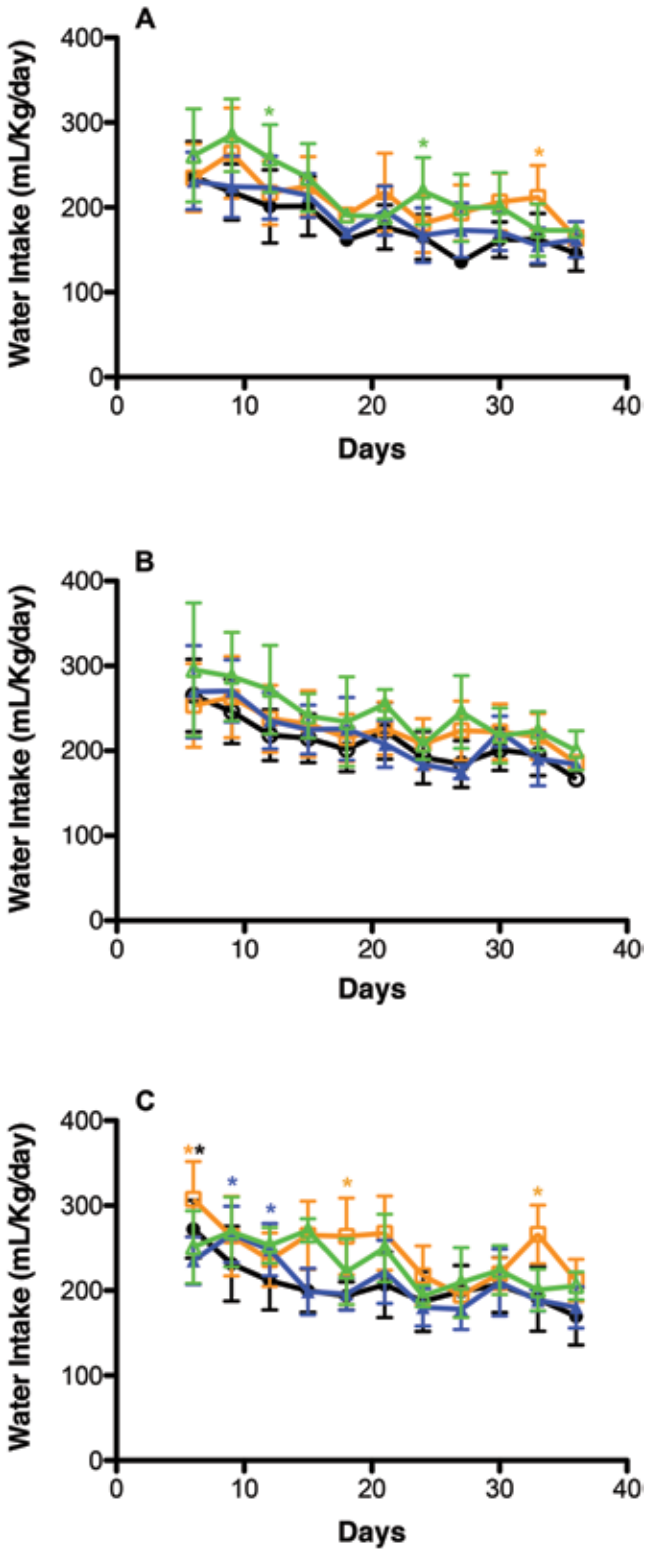

Figure 3. Water intake in mL/kg/day (mean ± 1 SD; $n = 6$ cages treated as a unit each, each cage consisting of 5 mice) of male and female BALB/c (A), C3H (B), and B6 (C) given acidified water ad libitum and maintained on either control CWF (male, solid black circles; female, open amber squares) or experimental bLAD (male, solid blue triangles; female, open green triangles) lighting conditions. Asterisks denote significant differences. Color of the asterisk denotes the group with the higher mean value.

Lighting type had a significant effect on weight when averaged across all time points ($P < 0.05$) for male BALB/c mice, male C3H mice, female C3H mice, and female B6 mice (Figure 2 and Table 2). However, no significant day:light interactions were found for weight between BALB/c, C3H, B6 mice housed under CWF conditions as compared with those housed under bLAD conditions for any strain and sex on specific days ($P > 0.05$).

Lighting type had a significant effect on water intake ($P < 0.05$) when averaged across all time points for male BALB/c mice (bLAD > CWF), female C3H mice (bLAD > CWF), and female B6 mice (CWF > bLAD) (Figure 3 and Table 3). However, when day:light interaction is taken into account, female BALB/c mice, male B6 mice, and female B6 mice had statistically significant effects ($P < 0.05$) on specific days for water intake. Further interrogating the day:light interaction, significant differences were seen on days 12, 24, and 33 between female BALB/c mice housed under the 2 different lighting conditions ($P < 0.05$; Table 4). On days 12 and 24, the bLAD group had a significantly higher mean for water intake than did the CWF group, but on day 33 the CWF group had a significantly higher mean than the bLAD group. Significant differences for water intake were seen on days 6, 9, and 12 between male B6 mice housed under the 2 different lighting conditions ($P < 0.05$; Table 4). On days 9 and 12, the bLAD group had a significantly higher mean for water intake than CWF group, but on day 6 the CWF group had a significantly higher mean than the bLAD group. Significant differences for water intake were seen on days 6, 18, and 33 between female B6 mice housed under the 2 different lighting conditions ($P < 0.05$; Table 4). For days 6, 18, and 33, the CWF group had a significantly higher mean for water intake than the bLAD group.

Lighting type had a significant effect on feed intake when averaged across all time points ($P < 0.05$) for male BALB/c mice, female C3H mice, male C3H mice, and male B6 mice (Figure 4 and Table 5). However, when the day:light interaction is taken into account, only male C3H mice were statistically different for feed intake on specific days. The bLAD group means were always significantly higher than those of the CWF group on days 6, 21, 27, 30, and 36 for feed intake ($P < 0.05$) (Table 6).

Serum blood chemistry mean values and standard deviation for both males and females of each strain (BALB/c, C3H, and B6) under each lighting condition are shown in Table 7. Significant differences were found for male C3H cholesterol ($P = 0.0409$; CWF > bLAD), female BALB/c total protein ($P = 0.0347$; bLAD > CWF), and female B6 phosphorus ($P = 0.0163$; bLAD > CWF). Complete blood count mean values and standard deviation for both males and females of each strain (BALB/c, C3H, and B6) and lighting condition are shown in Table 8. The only statistically significant effect was the neutrophil count of C3H females ($P = 0.0378$; bLAD > CWF).

Chemistry values omitted from the statistical analysis were albumin, globulin, total bilirubin, conjugated bilirubin, bicarbonate, potassium, sodium, chloride, unconjugated bilirubin, albumin/globulin ratio, BUN/Creatinine ratio and sodium/potassium ratio. Albumin and globulin were omitted as they are part of the total protein. In addition, little variation was observed among the values (albumin: $n = 320$, mean 2.5 g/dL, maximum 3.5 g/dL, minimum 1.5 g/dL, standard deviation 0.25 g/dL; globulin: $n = 320$, mean 1.85, maximum 2.8 g/dL, minimum 1.2 g/dL, standard deviation 0.21 g/dL). Little variation was seen among all values for total bilirubin, conjugated bilirubin, and unconjugated bilirubin: $n = 320$, mean 0.19 mg/dL, 0.01 mg/dL 0.18 mg/dL; maximum 0.9 mg/dL, 0.5 mg/dL, 0.4 mg/dL; minimum 0.1 mg/dL, 0 mg/dL, 0.1 mg/dL; standard deviation 0.07 mg/dL,

Table 3. Water intake, light, and day:light interactions

		CWF		bLAD		F p	
Strain	**Sex**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Light**	**Day:Light**
BALB/c	Male	178.8	41.8	190.4	39.8	9.02 **0.0133***	1.07 0.3899
BALB/c	Female	209.8	44.9	216.8	51.5	2.28 0.1621	2.63 **0.0069***
C3H	Male	210.7	40.2	217.3	43.7	3.57 0.0880	1.32 0.2328
C3H	Female	225.8	40.8	243.6	53.5	11.96 **0.0061***	0.72 0.7063
C57BL/6	Male	206.2	42.6	209.3	41.3	0.57 0.4680	2.53 **0.0093***
C57BL/6	Female	246.8	48.2	231.8	43.8	10.91 **0.0080***	3.62 **0.0004***

This table displays means and standard deviations averaged over the entire time period. Significant differences marked by (*) denote light effects on water intake over the entire period as well as significant differences for day:light interactions.

Table 4. Water intake individual day:light interactions

Water intake			BALB/c females		C57BL/6 males		C57BL/6 females	
Effect	Day	DF	t Value	Pr > |t|	t Value	Pr > |t|	t Value	Pr > |t|
Day:Light	6	100	−1.74	0.0846	2.73	**0.0074***	3.75	**0.0003***
Day:Light	9	100	−1.37	0.1738	−2.56	**0.0121***	−0.31	0.757
Day:Light	12	100	−2.61	**0.0103***	−2.7	**0.0082***	−1.12	0.2633
Day:Light	15	100	−0.66	0.5115	0.08	0.9343	−0.37	0.7111
Day:Light	18	100	0.03	0.9755	−0.15	0.8813	2.77	**0.0067***
Day:Light	21	100	1.89	0.061	−1.11	0.27	1.15	0.2518
Day:Light	24	100	−2.46	**0.0156***	0.51	0.612	1.6	0.1128
Day:Light	27	100	−0.38	0.7011	1.55	0.1238	−0.98	0.3306
Day:Light	30	100	0.38	0.7059	−0.23	0.8211	−0.27	0.7857
Day:Light	33	100	2.49	**0.0146***	0.13	0.8934	4.33	**<0.0001***
Day:Light	36	100	−0.57	0.5714	−0.77	0.4413	0.41	0.682

This table displays the statistical results for mice that had significant differences on certain days when day and light is taken into account as an interaction. Significant differences are denoted by (*).

Table 5. Food intake, light, and day:light interactions

		CWF		bLAD		F p	
Strain	**Sex**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Light**	**Day:Light**
BALB/c	Female	180.70	22.42	183.02	24.11	4.65 0.0564	1.34 0.2224
BALB/c	Male	175.75	19.00	180.97	21.06	16.59 **0.0022***	1.14 0.3407
C3H	Female	177.83	12.18	183.13	9.94	33.35 **0.0002***	1.47 0.1628
C3H	Male	175.75	11.99	183.76	13.59	50.33 **0.0001***	2.04 **0.0366***
C57BL/6	Female	185.01	13.33	187.80	13.27	4.33 0.0640	1.28 0.2522
C57BL/6	Male	160.11	13.49	164.72	12.72	19.95 **0.0012***	0.92 0.5211

This table displays means and standard deviations averaged over the entire time period. Significant differences marked by (*) denote light effects on food intake over the entire period as well as significant differences for day*light interactions.

0.04 mg/dL, 0.05 mg/dL; respectively. Bicarbonate was omitted from statistical analysis because it is useful mostly in understanding the blood-gas dynamics, which was not within scope of this study. Chloride, sodium, and potassium were omitted because insufficient numbers were obtained from serum chemistries for statistical analysis. Some samples collected were not large enough for analysis and therefore required dilution, resulting in the loss of chloride, sodium, and potassium values.

Percentages of nesting type (Table 9) and nest location (Table 10) were calculated based on a total of 66 events among the strain, sex, and lighting condition (11 nesting events for 6 groups), omitting one instance of male B6 under CWF conditions because inadequate nesting material had been provided. Percentages of nest types were similar between lighting conditions. BALB/c mice tended to vary its nesting type between a dome appearance and a bowl appearance. C3H and B6 strains made nests that were primarily bowl or cup shaped. Only males were observed to make flat nests. Among all strains and sexes, regardless of lighting condition, the mice preferred to make nests toward the front of the cage (that is away from the vent and toward the entry of light into the cage). We observed only 2 instances of nests made in the middle of the cage.

Discussion

Light has a major role in regulating metabolic and physiologic homeostasis. This study focused on the welfare of mice housed in individually ventilated cages under different lighting conditions. Previous studies described the effects of light in

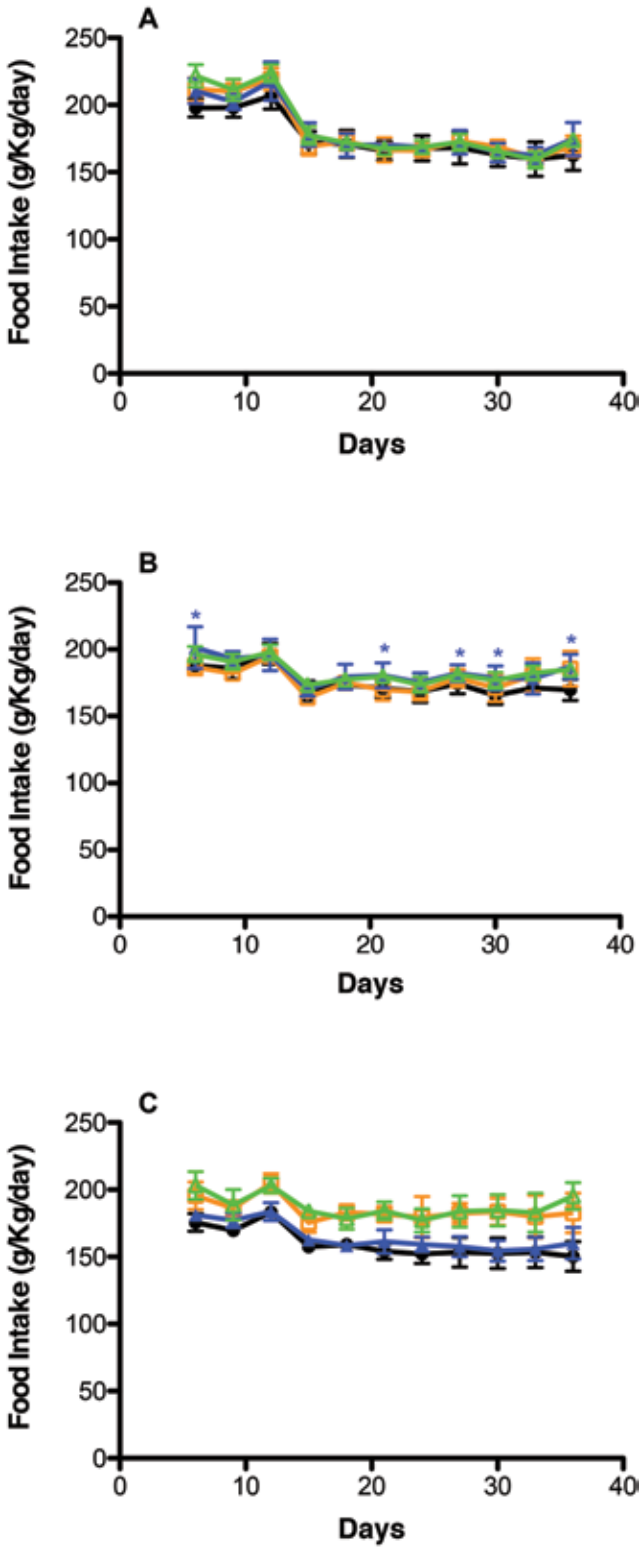

Figure 4. Food intake in g/kg/day (mean ± 1 SD; $n = 6$ cages treated as a unit each, each cage consisting of 5 mice) of male and female BALB/c (A), C3H (B), and B6 (C) mice fed normal chow ad libitum and maintained on either control CWF (male, solid black circles; female, open amber squares) or experimental bLAD (male, solid blue triangles; female, open green triangles) lighting conditions. Asterisks denote significant differences. Color of the asterisk denotes the group with the higher mean value.

the blue-appearing portion of the visible spectrum (similar to natural sunlight) on rodents.[7,19] We theorize that exposure to more natural daytime lighting conditions is better for animal welfare and physiology. The current study examined the influence of lighting type on a variety of parameters related to animal welfare. These parameters were body weight, feed intake, water intake, blood chemistry, complete blood count, nesting type, and nesting location.

Table 6. Food intake individual day:light interactions

Food intake			C3H males	
Effect	Day	DF	t Value	Pr > |t|
Day:Light	6	100	−3.58	**0.0005***
Day:Light	9	100	−1.91	0.0587
Day:Light	12	100	0.21	0.8359
Day:Light	15	100	−0.24	0.8095
Day:Light	18	100	−1.5	0.1359
Day:Light	21	100	−2.58	**0.0115***
Day:Light	24	100	−1.74	0.0844
Day:Light	27	100	−2.11	**0.0375***
Day:Light	30	100	−3.47	**0.0008***
Day:Light	33	100	−1.89	0.0613
Day:Light	36	100	−4.71	**<0.0001***

This table displays the statistical results for mice that had significant differences on certain days when day and light is taken into account as an interaction. Significant differences are denoted by (*).

Our investigation occurred during the growth phase of mice. Mice were exposed to lighting conditions from weaning age (4 wk of age) through 36 d after weaning age. No strain and sex of mice had significantly different body weights during the 36 d growth phase after weaning age when the 2 lighting conditions were compared on specific days. However, when averaged over the entire 36 d growth phase, most of the groups were significantly different when comparing body weights, with the exception of BALB/c females and B6 males, with bLAD mice weighing significantly less on average than their CWF counterparts. However, these significant differences should be viewed with caution, as the magnitude of the differences are minimal, with the most significant difference being 0.6 g. The strain with the largest disparity is the C3H group, which is consistent with previous research using mice housed on a static system.[7] Furthermore, both male and female C3H groups showed a plateau of the weight gain trajectory that occurred sooner in the bLAD group than in the CWF group. The trajectory of weight gain among BALB/c and B6 mice were similar between lighting groups.

Significant differences in water intake, averaged over the 36-day test period, were detected in BALB/c males, C3H females, and B6 females when comparing groups with different lighting conditions. When both light and day interactions are considered, BALB/c females, B6 males, and B6 females show significant differences on certain days. However, despite statistical significance, the differences are minimal and plots intersect at multiple time points, thus undermining the potential clinical significance of these effects. The general trend of lower water intake over time in all strains was most likely due to a greater weight gain over time relative to overall water intake. This result is different from previous reports of bLAD on water intake in which both male and female C3H mice had lower water intake under bLAD conditions.[7]

Significant differences in feed intake occurred in BALB/c males, C3H females, C3H males, and B6 males as a function of light when comparing means over the 36 d period. However, when comparing day:light interactions, only C3H males were

Table 7. Chemistries

		CWF			bLAD			
Strain/Sex	**Variable**	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	***P*(W)**
BALB/c male	**ALP (U/L)**	28	98.68	16.33	28	103.89	18.96	0.0742
	AST (U/L)	28	124.54	291.28	28	91.79	95.88	0.7442
	ALT (U/L)	28	90.00	280.31	28	50.86	79.17	0.9935
	CK (U/L)	28	489.82	1095.60	28	596.68	2113.53	0.6717
	Tot_Protein (g/dL)	28	4.29	0.38	28	4.38	0.37	0.3732
	BUN (mg/dL)	28	35.14	40.32	28	27.61	17.83	0.3407
	Creatinine (mg/dL)	28	0.38	1.37	28	0.16	0.46	0.7224
	Cholesterol (mg/dL)	28	89.14	18.53	28	90.71	17.57	0.9870
	Glucose (mg/dL)	28	251.61	72.18	27	243.78	53.71	0.9133
	Calcium (mg/dL)	28	7.86	0.68	28	8.16	0.68	0.3487
	Phosphorus (mg/dL)	28	10.23	6.24	28	8.87	2.82	0.5147
C3H male	**ALP (U/L)**	25	95.80	14.88	27	93.67	16.95	0.3496
	AST (U/L)	25	69.72	50.30	27	90.70	81.29	0.2729
	ALT (U/L)	25	49.16	44.73	27	67.44	75.01	0.3012
	CK (U/L)	25	176.56	196.82	27	135.44	157.77	0.7499
	Tot_Protein (g/dL)	25	4.66	0.33	27	4.59	0.22	0.5558
	BUN (mg/dL)	25	26.40	6.19	27	25.59	5.50	0.5657
	Creatinine (mg/dL)	25	0.10	0.07	27	0.09	0.10	0.2367
	Cholesterol[a] (mg/dL)	**25**	**130.36**	**15.73**	**27**	**118.63**	**17.60**	**0.0409**
	Glucose (mg/dL)	25	293.96	65.62	27	261.63	58.84	0.0801
	Calcium (mg/dL)	25	8.60	0.73	27	8.59	0.57	0.6222
	Phosphorus (mg/dL)	25	8.88	1.59	27	8.84	1.35	0.8553
C57BL/6 male	**ALP (U/L)**	28	76.68	13.98	28	79.71	14.44	0.5518
	AST (U/L)	28	62.86	60.79	28	79.46	64.49	0.0985
	ALT (U/L)	28	46.14	96.31	28	63.79	75.57	0.2358
	CK (U/L)	28	120.46	183.45	28	127.61	120.90	0.1272
	Tot_Protein (g/dL)	28	4.30	0.32	28	4.41	0.30	0.0922
	BUN (mg/dL)	28	23.93	5.69	28	23.14	4.49	0.5034
	Creatinine (mg/dL)	28	0.08	0.06	28	0.08	0.05	0.5978
	Cholesterol (mg/dL)	28	75.39	19.20	28	84.25	26.55	0.3457
	Glucose (mg/dL)	28	295.75	54.18	28	284.18	52.14	0.4115
	Calcium (mg/dL)	28	8.35	0.42	28	8.39	0.45	0.5818
	Phosphorus (mg/dL)	28	7.55	1.30	28	7.61	1.60	0.8256
BALB/c female	**ALP (U/L)**	23	111.96	14.48	24	113.67	15.78	0.6569
	AST (U/L)	23	77.61	44.65	24	95.13	94.81	0.8489
	ALT (U/L)	23	43.00	50.19	24	72.96	110.54	0.1692
	CK (U/L)	23	127.65	121.77	24	113.88	79.12	0.8489
	Tot_Protein[a] (g/dL)	**23**	**4.12**	**0.38**	**24**	**4.30**	**0.36**	**0.0347**
	BUN (mg/dL)	23	24.26	5.86	24	24.17	6.13	0.8650
	Creatinine (mg/dL)	23	0.04	0.05	24	0.04	0.06	1.0000
	Cholesterol (mg/dL)	23	68.43	19.21	24	73.96	19.29	0.2269
	Glucose (mg/dL)	23	223.61	39.08	24	221.13	42.42	0.8324
	Calcium (mg/dL)	23	7.75	0.70	24	7.96	0.88	0.0729
	Phosphorus (mg/dL)	23	8.00	1.48	24	7.78	1.48	0.7428
C3H female	**ALP (U/L)**	26	122.27	19.24	27	118.44	12.73	0.3065
	AST (U/L)	26	107.15	92.07	27	93.78	101.50	0.7433
	ALT (U/L)	26	61.00	58.60	27	49.07	49.63	0.4851
	CK (U/L)	26	121.04	123.15	27	154.00	163.69	0.8111
	Tot_Protein (g/dL)	26	4.44	0.52	27	4.46	0.27	0.4799
	BUN (mg/dL)	26	23.12	5.11	27	23.11	4.96	0.8452
	Creatinine (mg/dL)	25	0.07	0.07	27	0.06	0.07	0.4250
	Cholesterol (mg/dL)	26	98.23	18.68	27	94.22	19.29	0.5418
	Glucose (mg/dL)	26	239.85	57.56	27	268.59	70.82	0.2218
	Calcium (mg/dL)	26	8.48	1.03	27	8.70	0.85	0.4786

Table 7. Continued

Strain/Sex	Variable	CWF N	CWF Mean	CWF Std Dev	bLAD N	bLAD Mean	bLAD Std Dev	*P*(W)
	Phosphorus (mg/dL)	26	8.25	1.38	27	8.80	1.66	0.4317
C57BL/6 female	**ALP (U/L)**	27	108.41	14.86	29	106.45	25.27	0.8065
	AST (U/L)	27	69.04	36.00	29	71.07	38.61	0.7196
	ALT (U/L)	27	35.93	28.29	29	39.45	43.69	0.8380
	CK (U/L)	27	102.74	125.63	29	98.72	66.14	0.4298
	Tot_Protein (g/dL)	27	4.39	0.19	29	4.53	0.46	0.6574
	BUN (mg/dL)	27	26.41	9.68	29	27.28	7.39	0.2757
	Creatinine (mg/dL)	27	0.08	0.12	29	0.08	0.09	0.7367
	Cholesterol (mg/dL)	27	65.26	14.15	29	67.83	16.54	0.4682
	Glucose (mg/dL)	27	274.37	37.09	29	268.28	59.41	0.8575
	Calcium (mg/dL)	27	8.53	0.40	29	8.72	0.78	0.9345
	Phosphorus[a] (mg/dL)	**27**	**7.51**	**1.36**	**29**	**8.41**	**1.76**	**0.0163**

[a]Indicates significant differences

Summary of biochemistry results of 3 strains of mice (BALB/c, C3H, and B6) and both sexes maintained on IVC system under 2 different lighting conditions (bLAD or CWF).

significantly different on specific days: 6, 21, 27, 30, and 36. C3H males housed under bLAD conditions consumed more feed per kilogram of body weight over the entire study as compared with CWF mice ($P < 0.05$). This finding differs from previously published research that found the opposite; C3H males housed under CWF conditions consumed more feed than C3H housed males under bLAD conditions.[7] The basis for greater food consumption by C3H mice under bLAD conditions is unknown. Furthermore, for unknown reasons, in the current study all strains and sexes showed a sharp decline in feed intake between days 9 and 12, after which feed intake began to level off. Much like water intake, this could be an effect of body weight increasing to a greater degree than did feed intake. The only strain that differed in this regard between sexes was B6. Females tended to consume more feed over time than B6 males.

When assessing human and animal health, a rapid and accepted way to evaluate how the body is functioning and regulating is through an evaluation of blood. Therefore, to evaluate any effects different lighting conditions have on homeostasis, blood was drawn via cardiocentesis after the 36 d test period. A few significant differences were noted in the complete blood serum chemistry panel. In C3H males, those housed under bLAD conditions had significantly lower cholesterol than did those housed under CWF conditions. These findings are similar to those found previously in mice housed under similar conditions in static caging.[7] Total protein was significantly higher in BALB/c females under bLAD conditions as compared with CWF conditions. Phosphorus levels were significantly higher in B6 females under bLAD conditions as compared with under CWF conditions. Although the values differ statistically, they are similar to published ranges and therefore we consider them to be clinically insignificant.[4,5,13,20,24,25,27,31] Thus, neither lighting condition appeared to influence normal serum chemistry of the mice dramatically. We also assessed the health of the animals through a complete blood count. However, in this study the only statistically significant effect was that C3H females housed in bLAD conditions had higher neutrophil counts than those housed under CWF conditions. These animals appeared healthy and their values were similar to previously reported hematology ranges.[4,5,23-25,27] The lack of significant differences in neutrophil-lymphocyte ratio levels and glucose levels suggests neither lighting condition presents a more stressful condition than the other for both sexes of these 3 strains of mice.[17]

We also studied the effect of these lighting conditions on nesting behavior. A previous experiment categorized types of nests made by mice as flat, bowl (or cup), incomplete dome, and dome.[16] We were unable to perform statistical analysis of this data due to low statistical power, but percentages of each nest time and location were calculated for 66 events distributed among all strains and sexes. Lighting condition did not appear to effect the type of nest made or its location in the cage. This finding is consistent with a previous study that investigated nesting as a measure of maternal behavior in ICR mice.[19] That study also found no significant difference found in nest type between bLAD and fluorescent lighting.[19] Furthermore, in the current study, BALB/c male and female mice housed under CWF conditions made dome type nests in numbers that were approximately equal to that of bowl type nests, which were the dominant type of nest built by the other 2 strains. In addition, males, with the exception of BALB/c under CWF conditions and B6 under bLAD conditions, had at least one instance of making a completely flat nest. This was not observed in any female mice. Among all strains and sexes, the mice preferred to build nests toward the front of the cage (that is where light enters the cage and away from the ventilation inflow port). BALB/c females and males, except for one instance B6 females housed under CWF conditions, under both light conditions were the only strain to build nests toward the back of the cage. Mice did not build nests in the middle of the cage except for one instance in B6 male housed under CWF and one instance in BALB/c female housed under CWF conditions. Ventilation or lighting entering the cage could influence where in the cage mice build their nests. Building nests away from the vent could be a coping mechanism to reduce cold stress.[11] Additional studies are needed to characterize the role of light and air flow on nest location and quality. Furthermore, one study reported that C57BL/6 mice exposed to either bLAD or CWF showed no behavioral differences when performing in Y-maze test, object recognition test, tail suspension test, and open field test.[12] In the present study and in a few previously mentioned studies,[12,19] no major differences were seen in behavior between lighting conditions. Other behavioral tests could be affected by lighting, especially in melatonin producing strains of mice.

Overall, the results of this experiment using an IVC system and these 3 strains of male and female mice do not support the

Table 8. Complete blood counts

		CWF			bLAD			
Strain/Sex	**Variable**	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**p(W)**
BALB/c male	**Neutrophil (cells/µL)**	15	677.33	352.26	15	776.60	357.62	0.4613
	WBC (K/µL)	15	3.57	2.29	15	3.71	1.90	0.5793
	RBC (M/µL)	15	9.18	0.51	15	9.19	0.66	0.1515
	HGB (g/dL)	15	13.91	0.78	15	13.95	0.95	0.1350
	Lymphocyte (cells/µL)	15	2762.13	1898.65	15	2784.20	1553.80	0.7736
	NL_Ratio	15	0.26	0.07	15	0.30	0.10	0.2308
	HCT (%)	15	43.93	2.13	15	43.97	2.96	0.2231
	Monocyte (cells/µL)	15	79.20	43.40	15	98.07	65.36	0.4427
	Eosinophil (cells/µL)	15	53.27	48.25	15	51.47	25.33	0.4606
	Basophil (cells/µL)	15	1.47	3.87	15	2.87	4.21	0.3270
C3H male	**Neutrophil (cells/µL)**	14	1158.14	407.83	16	910.56	302.70	0.0878
	WBC (K/µL)	14	3.91	0.98	16	3.27	1.04	0.0713
	RBC (M/µL)	14	8.15	0.39	16	7.77	1.64	0.5650
	HGB (g/dL)	14	12.96	0.62	16	12.40	2.74	0.6502
	Lymphocyte (cells/µL)	14	2592.36	737.23	16	2241.06	800.84	0.1400
	NL_Ratio	14	0.45	0.15	16	0.43	0.15	0.6806
	HCT (%)	14	42.12	2.37	16	41.04	8.40	0.9179
	Monocyte (cells/µL)	14	101.29	47.47	16	73.75	39.42	0.1070
	Eosinophil (cells/µL)	14	50.29	28.91	16	41.63	15.59	0.4124
	Basophil (cells/µL)	14	5.14	6.89	16	2.44	4.40	0.2735
C57BL/6 male	**Neutrophil (cells/µL)**	25	1040.08	516.83	22	947.73	582.75	0.3923
	WBC (K/µL)	25	5.17	1.87	22	4.70	2.19	0.3162
	RBC (M/µL)	25	8.87	0.47	22	8.75	0.36	0.5892
	HGB (g/dL)	25	13.14	0.67	22	12.98	0.53	0.7740
	Lymphocyte (cells/µL)	25	3914.44	1352.33	22	3555.64	1597.26	0.4281
	NL_Ratio	25	0.26	0.08	22	0.31	0.27	0.8904
	HCT (%)	25	45.44	2.68	22	44.30	1.90	0.2185
	Monocyte (cells/µL)	25	101.80	66.92	22	89.82	62.89	0.4656
	Eosinophil (cells/µL)	25	109.68	60.47	22	108.91	68.60	0.8820
	Basophil (cells/µL)	25	2.16	4.12	22	2.41	5.56	0.8126
BALB/c female	**Neutrophil (cells/µL)**	13	804.23	364.21	18	799.00	358.95	0.9842
	WBC (K/µL)	13	3.78	1.45	18	3.88	1.75	1.0000
	RBC (M/µL)	13	8.68	1.81	18	9.08	0.46	0.7358
	HGB (g/dL)	13	13.39	3.35	18	14.13	0.66	0.5385
	Lymphocyte (cells/µL)	13	2800.69	1019.50	18	2910.89	1362.71	0.9525
	NL_Ratio	13	0.28	0.06	18	0.28	0.07	0.8414
	HCT (%)	13	41.33	8.65	18	43.39	2.14	1.0000
	Monocyte (cells/µL)	13	83.00	59.98	18	86.72	66.73	0.9367
	Eosinophil (cells/µL)	13	94.15	69.89	18	83.67	63.09	0.8272
	Basophil (cells/µL)	13	2.77	5.28	18	3.22	5.87	0.9383
C3H female	**Neutrophil[a] (cells/µL)**	**18**	**791.56**	**372.28**	**17**	**1131.82**	**557.71**	**0.0378**
	WBC (K/µL)	18	2.99	1.19	17	3.64	1.57	0.2694
	RBC (M/µL)	18	8.14	0.27	17	7.92	1.64	0.4726
	HGB (g/dL)	18	13.03	0.59	17	12.65	2.51	0.9215
	Lymphocyte (cells/µL)	18	2063.78	826.13	17	2365.71	1093.87	0.4433
	NL_Ratio	18	0.40	0.13	17	0.51	0.17	0.0502
	HCT (%)	18	41.96	1.84	17	40.97	8.39	0.4824
	Monocyte (cells/µL)	18	96.83	57.80	17	99.06	58.69	0.8441
	Eosinophil (cells/µL)	18	41.33	42.37	17	41.00	39.69	0.3169
	Basophil (cells/µL)	18	1.06	3.08	17	3.65	5.12	0.0849
C57BL/6 female	**Neutrophil (cells/µL)**	21	951.95	406.27	20	914.30	450.38	0.6505
	WBC (K/µL)	21	4.77	1.58	20	4.54	1.48	0.5179
	RBC (M/µL)	21	8.74	0.45	20	8.79	0.47	0.9897

Table 8. Continued

Strain/Sex	Variable	CWF N	CWF Mean	CWF Std Dev	bLAD N	bLAD Mean	bLAD Std Dev	p(W)
	HGB (g/dL)	21	13.14	0.66	20	13.27	0.75	0.9069
	Lymphocyte (cells/µL)	21	3592.62	1240.15	20	3436.10	1147.30	0.6138
	NL_Ratio	21	0.27	0.10	20	0.27	0.10	0.8764
	HCT (%)	21	43.97	2.38	20	44.28	2.10	0.8968
	Monocyte (cells/µL)	21	115.95	51.76	20	107.45	74.99	0.2578
	Eosinophil (cells/µL)	21	100.71	58.89	20	83.45	56.86	0.2369
	Basophil (cells/µL)	21	5.38	4.93	20	5.05	7.06	0.6656

[a]Indicates significant differences
Summary of hematology results of 3 strains of mice (BALB/c, C3H, and B6) and both sexes maintained on IVC system under 2 different lighting conditions (bLAD or CWF).

Table 9. Nest type

Strain	Sex	Lighting condition	Dome	Bowl or cup	Flat
BALB/c	Male	CWF	52%	48%	0%
BALB/c	Male	bLAD	47%	50%	3%
C3H	Male	CWF	6%	92%	2%
C3H	Male	bLAD	3%	95%	2%
C57BL/6	Male	CWF	6%	91%	3%
C57BL/6	Male	bLAD	0%	100%	0%
BALB/c	Female	CWF	56%	44%	0%
BALB/c	Female	bLAD	38%	62%	0%
C3H	Female	CWF	3%	97%	0%
C3H	Female	bLAD	3%	97%	0%
C57BL/6	Female	CWF	2%	98%	0%
C57BL/6	Female	bLAD	0%	100%	0%

Summary of nest type results of 3 strains of mice (BALB/c, C3H, and B6) and both sexes maintained on IVC system under 2 different lighting conditions (bLAD or CWF).

Table 10. Nest location

Strain/sex/lighting condition	Front right	Front middle	Front left	Middle right	Middle center	Middle left	Rear right	Right center	Rear Left
BALB/c, Male, CWF	29%	47%	8%	0%	0%	0%	14%	1%	1%
BALB/c, Male, bLAD	15%	71%	8%	0%	0%	0%	0%	3%	3%
C3H, Male, CWF	21%	21%	58%	0%	0%	0%	0%	0%	0%
C3H, Male, bLAD	33.3%	33.3%	33.3%	0%	0%	0%	0%	0%	0%
C57BL/6, Male, CWF	12%	42%	45%	0%	0%	1%	0%	0%	0%
C57BL/6, Male, bLAD	23%	45%	32%	0%	0%	0%	0%	0%	0%
BALB/c, Female, CWF	21%	45%	21%	2%	0%	0%	0%	9%	2%
BALB/c, Female, bLAD	14%	74%	9%	0%	0%	0%	3%	0%	0%
C3H, Female, CWF	36%	8%	56%	0%	0%	0%	0%	0%	0%
C3H, Female, bLAD	47%	14%	39%	0%	0%	0%	0%	0%	0%
C57BL/6, Female, CWF	26%	44%	29%	0%	0%	0%	0%	0%	1%
C57BL/6, Female, bLAD	9%	67%	24%	0%	0%	0%	0%	0%	0%

Summary of nest location results of 3 strains of mice (BALB/c, C3H, and B6) and both sexes maintained on IVC system under 2 different lighting conditions (bLAD or CWF).

idea that one lighting condition is superior for the welfare of mice. However, as demonstrated by our laboratory and others,[7-10,14,15,29,30] lighting conditions clearly influence research outcomes. Such evidence is seen in the current study with regard to significant differences in a number of parameters in mice maintained under either CWF or bLAD lighting conditions, including body weight, food intake, water intake, and some hematology and biochemical values. The limitations of this study include the need to change cages every 3 d, which could be an acute stressor in mice and therefore have metabolic and physiologic consequences. In addition, blood analysis would be more accurately accomplished at point-of-care, although refrigerated blood samples and frozen serum samples have been shown to give similar results to those of blood samples run at point-of-care.[18,22] In future experiments, conducting the study for longer periods, as done previously, may reveal additional or more pronounced significant changes.[7] Further studies in other species are warranted to determine whether potential welfare concerns arise under bLAD compared with CWF lighting conditions.

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