# The effect of relative humidity on water intake of C57BL/6J mice housed under conditions of controlled relative humidity at cage level

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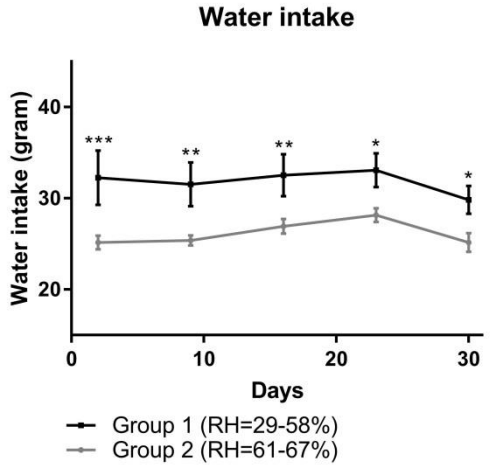
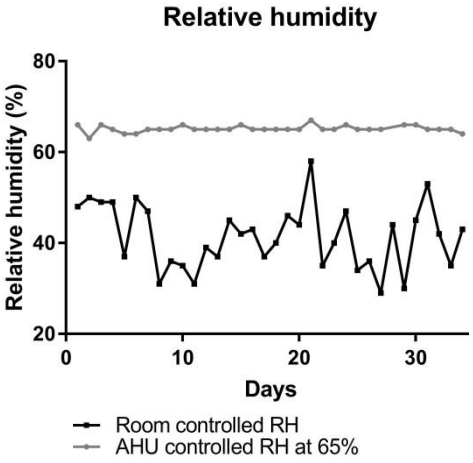
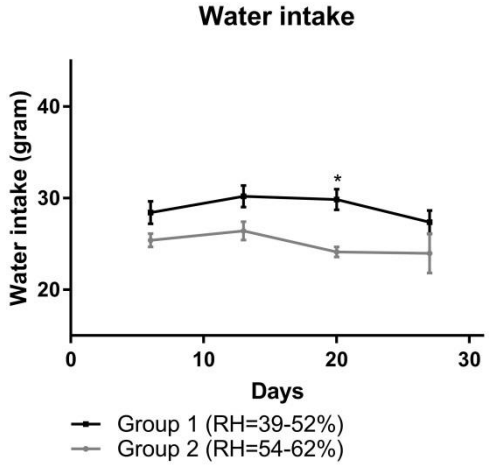
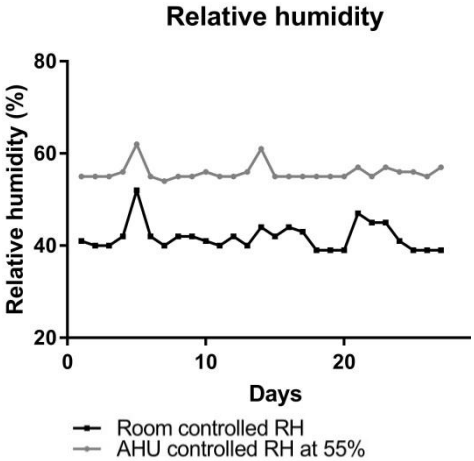
Aim of study: Food and water intake of laboratory animals are parameters measured in various research fields such as diabetes, obesity and behaviour. The housing environment can affect these parameters. E.g. it has been shown that rats housed under low relative humidity (RH) had a higher food intake than rats housed at high RH[1] and housing conditions can affect the water balance of mice[2]. In the current study we aimed to investigate the effect of relative humidity on water intake of mice housed at RH controlled steadily at cage level when compared to mice housed in cages with RH controlled less steadily at room level. To evaluate the effect of RH on water intake of mice, controlled RH at three different levels was compared with RH controlled at room level. The rationale for the study was to evaluate potential effects of RH on water intake, which can potentially affect reproducibility in experimental results.

Material and Methods: To investigate the effect of controlled RH on murine water intake an air handling unit (AHU) capable of controlling RH (ScanClime®) was used (Group 2). This AHU accurately controlled RH in IVC systems from Tecniplast, at three different levels within regulatory requirements (45%, 55% and 65%). A setup using the same IVC cages connected to an AHU not controlling humidity was used for comparison (Group 1). The latter system was subject to RH controlled less stable at room level. The study was performed over three months. For the first month RH was set to 65%, the second 55% and the last 45%. Daily RH readings were recorded from inside the cage, the AHUs and the holding room. Two groups with a mix of female and male C57BL/6J mice were compared (N=35) aged 2-6 months at the beginning of the study. The age distribution was equal in the two groups. 2-4 animals were housed in each cage. All mice maintained under the ScanClime were first acclimatised to the unit for two months. Water intake was measured on a weekly basis as an average pr. mouse pr. cage. Due to bio-security restrictions the cages were cleaned on a Monday between 13h00-15h00, and to avoid double handling the animals and water bottles were weighed during this husbandry routine. This also reduced the accidental loss of water through excess moving of cages. To work out the water intake the bottles were weighed, and the following Monday they were weighed again; the difference was then divided by the number of animals in the cage to find the average intake per animal. The animals were weighed one Mondays as well. All animals were housed in IVC caging on aspen bedding with nesting material, cardboard tunnel and chew sticks that had been autoclaved. They were fed standard rodent chow ad libitum, and housed on a 12h day/night cycle. Both groups of animals were housed in the same room but were on different racks with different AHUs. The statistical analyses used were repeated measurements ANOVA (SAS Enterprise Guide 7.1). Daily welfare assessments were performed together with a thorough check at the weekly cage changes. The study was carried out under license No. X7069FDD2 issued by the Home Office UK.

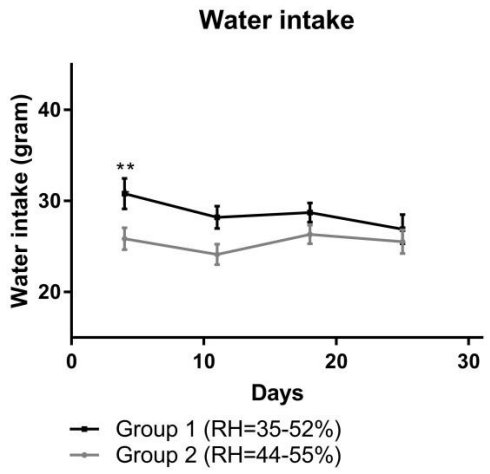
Results: The results of comparing a RH of 65% with room controlled RH of 29-58% showed that mice housed in the controlled environment drank significantly less during the one month test period. The same was shown for the months of testing RH at 55% and 45% compared to room controlled RH. Here one of the weekly water intake measures were significantly lower than the measures of mice housed under room controlled humidity in each month, respectively. See graphs 1, 3 and 5 below. When including age and gender to the analyses a significant difference in water intake was found between the two groups in the month were the ScanClime was set to 65% (data not shown). No differences were observed in body weight between the two groups throughout the study period (data not shown). During the experiment four animals, two from each group were taken out of the experiment due to different health issues.

**Table 1. Water intake and relative humidity over the three month testing period.**

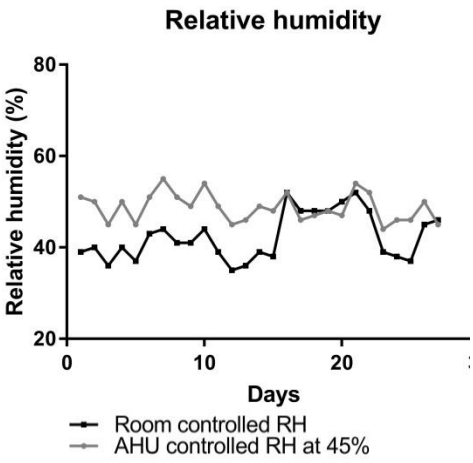
| | | |
|---|---|---|
| **Month 1: ScanClime set at 65%** | **1**   | **2**   |
| **Month 2: ScanClime set at 55%** | **3**   | **4**   |

**Month 3: ScanClime set at 45%**

**5**




**6**




Discussion: The ScanClime AHU used in this experiment was not set to dehumidify due to the installations available in the facility. As the dehumidification feature needs a cooling circuit in the facility. This can be seen in the two months were the RH is set to 55% and especially 45%. When the RH is higher in the room the ScanClime is not capable of lowering the RH. To really look more into the effect of steady RH on the animals, including their water intake, a future study using a unit set to dehumidify could be interesting. Future studies are generally warranted to explore the effects of RH on larger groups of animals and on different parameters perhaps food intake or other metabolic parameters. The challenge to reproduce study results in preclinical research has gained increasing focus over the past years[3]. A focus on stabilizing the environment of the animals or improving registration of the environmental parameters could be one way of approaching this challenge.

Conclusion: Our results suggest that RH can affect the water intake of C57BL/6J mice. When tightly controlling RH within regulatory requirements the mice drink significantly less than when the animals are housed under room controlled RH. To ensure accurate data results and reproducibility of studies, it can be of great value to steadily control the RH.

References:

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