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Original scientific article

Are female mice dehydrated during peak lactation? Effect of water and gel supplement on hydration parameters and water consumption in two strains of mice

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Summary

Mice (*Mus musculus*) have a high basal rate of metabolism which increases during pregnancy and lactation. During peak lactation, water intake amounts to up to 65 % of the bodyweight per day. Providing water in a bottle may pose a restriction of water intake and lead to dehydration during periods of high demand, such as peak lactation. To establish if female mice are able to sustain a physiological hydration status during peak lactation, a completely randomized factorial design study was conducted with 12 RjOrl:SWISS (SWISS) and 12 C57BL/6JRj (B6) six-week old female mice in breeding. Female mice were randomly assigned to one of three groups with different watering alternatives: water bottle (Standard, n=6); water bottle + sachet with 98 % water gel (Gel, n=6); or water bottle + water bowl (Bowl, n=6). Non-mated females, provided with water bottles, served as controls (n=6). Hydration parameters [total protein (TP), hemoglobin, hematocrit, serum osmolality (Osmol), blood urea nitrogen (BUN)] and magnesium were measured in blood before mating (Pre) and during peak lactation (Peak), and at the same time points in controls. Water bottles were weighed during lactation and body weights of females and litters recorded at weaning. Data were analyzed by parametric or non-parametric methods to evaluate effects of strain, group and time point. The hydration parameters and magnesium were mostly within normal ranges in all animals at Pre and Peak. TP was lower at Peak in all lactating groups compared to Controls and to Pre ($p<0.01$). Mice in group Bowl consumed 54 % less bottle water compared with Gel and Standard ($p<0.001$), had 34 % lower levels of BUN than Standard and Control ($p<0.01$) and 5 % lower serum osmolality at Peak than Pre ($p<0.01$).

Conclusion: Female mice are not dehydrated at peak lactation. However, they prefer to drink, and seemingly drink more water, from a bowl than from a bottle.

Introduction

Owing to their small body size, mice have a very high rate of mass-specific metabolism. Laboratory mice are generally provided with dry feed, containing approximately 10 % of water, and drinking water in bottles or automated water systems. Adequate water intake is essential for animal welfare, and restriction can reduce performance and increase the risk of diseases. During peak lactation, female mice can increase feed and water intake up to four times, leading to a water intake that amounts to approximately 60 % of their body weight per day (Murai et al. 2013). The watering systems for housing laboratory mice are considered to provide water *ad libitum*. Water bottles are either equipped with a sipper tube or a cap with a hole from which the water is licked. The volume of each lick depends on the diameter of the

orifice and the amount of air in the inverted water bottle, as was shown in rats (Weijnen 1998). In rabbits, drinking water from a nipple drinker has been shown to take longer and result in lower water intake compared with drinking from a bowl (Tschudin et al. 2011). Horses prefer to drink, and drink more, from a bucket compared with an automatic water bowl, even if the automatic water flow is meeting their normal drinking rate (Nyman and Dahlborn 2001).

A pilot study in lactating fancy mice showed that they prefer to drink from a bowl rather than a bottle. Adding a bowl to the cage increased water intake by 55 %, whereas bottle water intake decreased by 67 % (Hedenqvist et al. 2015). The preference for the bowl suggests that drinking from a bottle is more time consuming. At peak lactation when much time is spent on the large feed intake and tending to the pups, drinking from a bottle may pose a restriction for water intake, and thus be a welfare problem.

To test the hypothesis that lactating mice are dehydrated during peak lactation, a study was performed in mice of two strains. Water was provided in bottles, or additionally as gel or in a bowl during lactation. Parameters of hydration were measured before mating and at peak lactation, as was the bottle water consumption during lactation. Water as gel was included as part of the study because it would provide a more practical means of adding water compared with a bowl.

Material and Methods

Animals and housing

RjOrl:SWISS (SWISS) and C57BL/6JRj (B6) mice (12 females and 6 males per strain) were purchased from Janvier Labs (Le Genest-Saint-Isle, France) at 6 weeks of age (total n=42). The colony of origin was monitored according to FELASA recommendations (Mähler et al. 2014) and declared free from all listed agents. All mice were housed in IVC cages (Innocage®, Innovive, San Diego, USA) with 54 air changes per hour. The outside dimensions of the cage were: Length: 37.3 x Width: 23.4 x Height: 14.0 cm. Each cage contained corncob bedding, nesting material (shredded paper) and a ladder for enrichment (Figure 1A). The cage with enclosed contents was irradiated. Commercial irradiated pelleted food (SDS RM3, UK) and acidified water (2.5-3.0 pH) in pre-filled water bottles (300 ml, Aquavive®/Innovive) were provided *ad libitum*. The bottle was equipped with a cap which had a hole with a diameter of 1.2 mm (Figure 1B). The animal room was set on a 12:12 h light:dark cycle (light on 7.00 to 19.00), room temperature was maintained at 22 ± 2 °C with approximately 15 air changes per hour and with relative humidity of 52-55 %. After one week of acclimation, mice were handled daily for a week to minimize stress during blood sampling. Mice were removed from the cage with cupped hands or the cage ladder. The same techniques were used by the technical staff at weekly cage changes.

Nine females and nine males of the same strain were paired at the age of seven weeks. The hydration parameters were examined before mating and at peak lactation after the females had their second litter, since female mice usually have smaller first litters. When the first litter was born all the pups except two were removed and these pups and the males stayed with the females and were removed after the birth of the second litter. The age of the females was 16 to 19 weeks when the second litter was born.

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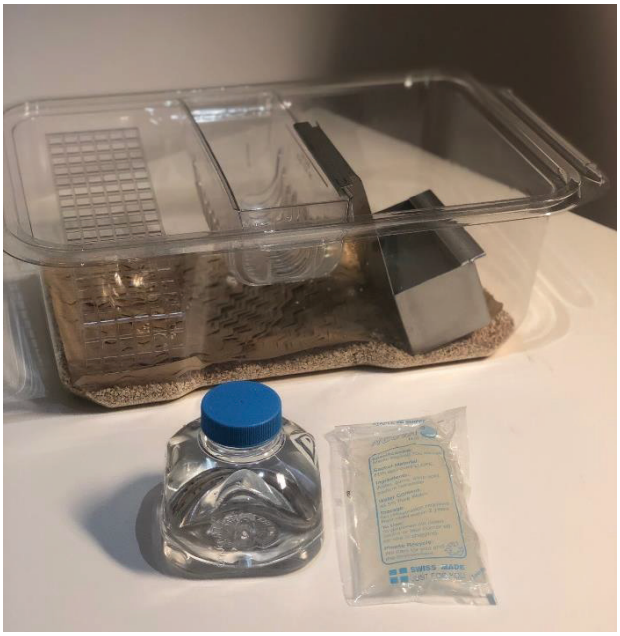

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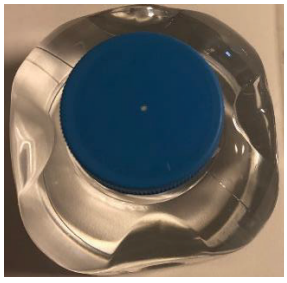

Figure 1. A. The IVC cage with nesting material, a ladder, and a stainless-steel water bowl attached. The gel sachet and the water bottle with the perforated cap are shown next to the cage. **B.** Close up of the bottle lid with the 1.2 mm large hole.

Experimental design

Female mice were assigned to one of three groups according to a completely randomized factorial design with two strains and three watering alternatives. The watering alternatives were:

1. Water bottle (Standard, n=6)
2. Water bottle plus one water gel sachet (Gel, n=6)
3. Water bottle plus a water bowl (Bowl, n=6)

Six female mice were left unmated (three SWISS and three B6), serving as controls. They were housed in groups of three in an Innocage® with a water bottle and no water supplements.

The gel and water bowl (Figure 1A) were added after the birth of the second litter. The water gel sachet size was 15 x 8 cm and was attached to the feeder by tape. The gel (NECTA H2O gel, Labodia, Switzerland) contained 98.5% pure water. The rest of the gel consisted of gums, ascorbic acid and sodium benzoate. Before putting the gel into the cage, one corner of the sachet was cut to make the gel accessible to the mice. The metal water bowl was attached to the feeder to avoid overturning. It was washed and filled with fresh tap water every other day. As the pups got older they were able to jump up on the bowl and drink from the water.

Blood sampling and analyses

For sampling, the vena saphena was punctured with a lancet and the blood collected, maximum 8 ml/kg BW in total, in serum and plasma vials (Microvette® CB 300 µl Serum, red US code and Microvette® CB 300 µl Lithium Heparin, green US code, Sarstedt, Nümbrecht Germany). The serum samples were left for one hour and then centrifuged for 10 minutes at 3000 rpm. As indicators of hydration status, total plasma protein (TP), hemoglobin (Hb), hematocrit (Htc), serum osmolality (Osmol) and blood urea nitrogen (BUN) were measured before mating (Pre) and at lactation day 14 (Peak). Magnesium (Mg) was also measured because previous cases of lethal intestinal pseudo obstruction in peak lactating female mice (Feinstein et al. 2008) had been diagnosed with hypermagnesemia (Tjäder et al. 2013). Blood was collected from control mice at the same time points.

Heparinized whole blood was analyzed with VetScan® VS2plus (Large Animal Profile reagent rotor, SweVet ABAXIS, Triolab AB, Mölndal, Sweden) and ABL90 FLEX (Radiometer, Bronshoj, Denmark). Serum osmolality was analyzed with Fiske Micro-Osmometer Model 210 (Fiske Associates, Norwood, Massachusetts, USA).

Other measurements

Water bottles were weighed on the first day after birth, before and after the weekly change of water bottles, and at weaning. Litter size and body weight of the pups and females were recorded at weaning.

Statistical analysis

InVivoStat was used for analyses (InVivoStat 1.3, 2020). Data was checked for normally distributed residuals with normal probability plots and for homogeneity of variance with predicted vs. residuals plots. Baseline data (Pre) were compared between strains by Student's t-test or Mann-Whitney test. Hydration parameters at Pre and Peak were analyzed using two-way repeated measures (RM) mixed model ANOVA, with group as main factor, time point as repeated factor and strain as blocking factor. Planned *post hoc* comparisons were performed without adjustment for multiplicity (LSD test) between timepoints within groups and between groups at Peak. Non-normally distributed data was compared between strains and groups at Peak by Mann-Whitney or Kruskal-Wallis test, and between time points within groups with Wilcoxon signed rank test. Consumption of bottle water during lactation was compared using a two-way ANOVA with strain and group as factors. The comparison included both the total water volume consumed, as well as the volume (ml) relative to body weight (g) of female and litter. A $p<0.05$ was set as the level for significance.

Ethics approval

The study was approved by the regional animal ethics committee in Lund, permit 5.8.18-17840/2017.

Results

Baseline

Table 1 shows baseline values before mating (Pre). SWISS mice had a higher level of TP than B6 (p=0.01). No other parameter differed between strains. TP levels were high in SWISS mice compared with reference values. Osmol values were high in both strains compared with reference values for mice. Some mice had higher levels of Mg than reference values.

Table 1. Data for hydration parameters and magnesium in blood in females of two mouse strains before mating (Pre). Data presented as mean ± SD (min-max). Reference values are presented for comparison.

Parameter at baseline (Pre)	RjOrl:SWISS (n=9)	C57BL/6JRj (n=9)	p-value (t-test)	Reference values*
Total protein (g/L)	66 ± 5 (58-79)	61 ± 3 (53-65)	0.01	44-58 32-59 45-62 45-48 41-59
Hemoglobin (g/L)	164 ± 9 (145-175)	160 ± 11 (129-170)	0.3	110-183 111-164
Hematocrit (%)	50 ± 3 (45-54)	49 ± 3 (39-52)	0.4	32-52 33-51 35-47
Serum osmolality (mOsmol/kg)	335 ± 9 (322-346)	336 ± 13 (319-364)	0.9	307-336
Blood urea nitrogen, BUN (mg/L)	194 ± 39 (140-250)	224 ± 49 (170-340)	0.1	193-434 141-353
Magnesium (mmol/L)	1.3 ± 0.3 (1.05-1.87)	1.3 ± 0.1 (1.08-1.40)	0.5	1-1.3

*Chollet et al. 2000; Otto et al. 2016; Santos et al. 2016; Serfilippi et al. 2003; Silverstein et al. 1961; Waymouth. 1970

Effect of lactation

Data at peak lactation are displayed in Table 2.

Strain (p=0.03) and time point (p<0.001) had a main effect on TP whereas group did not (p=0.3, two-way RM ANOVA). There was an interaction between group and time point (p=0.02). All lactating groups had lower TP at Peak than Pre, whereas Control did not differ (Standard: p=0.01, Gel: p=0.004, Bowl: p<0.001, Control: p=0.7). At Peak, all groups had lower TP than Control (Standard: p=0.006, Gel: p=0.01, Bowl: p=0.009).

Hb and Htc were not affected by strain (p=0.2), time point (p=0.5 and p=0.6 respectively) or group (p=0.7, two-way RM ANOVA). Osmol was not affected by strain (p=0.09) or group (p=0.2), but by time point (p=0.03). Bowl had a lower Osmol at Peak than Pre (p=0.006), whereas Standard (p=0.4), Gel (p=0.3) and Control Osmol did not change (p=0.8, two-way RM ANOVA).

Strain (p=0.9) and group (p=0.2) had no effect on BUN, whereas time point had an effect (p<0.001, two-way RM ANOVA). There was an interaction between group and time point (p=0.01). Control and Standard had higher levels of BUN at Peak than Pre (p<0.001) whereas Gel and Bowl had not (p=0.1 and 0.5). BUN was lower in Bowl than in Standard (p=0.005) and in Control (p=0.004) at Peak.

Strain and Group had no effect on Mg (p=1 and 0.4, respectively, two-way RM ANOVA), whereas time point had an effect (p=0.002). Mg was lower at Peak than Pre in Bowl and Control (p=0.01 and p=0.02, respectively). Groups did not differ at Peak.

Bottle water consumption and litter data

The absolute water consumption data was Log 10 transformed prior to analysis (two-way ANOVA). Strain had an effect on bottle water consumption; SWISS mice drank more than B6 (p<0.001). Group

Table 2. Data for hydration parameters and magnesium in blood in females of two mouse strains [RjOrl:SWISS and C57BL/6JRj] at peak lactation. Watering alternatives for lactating females: Standard = water bottle; Gel = water bottle + water gel sachet; Bowl = water bottle + water bowl. Control = non-lactating females, water bottle. Data presented as median (min-max), or mean ± SD (min-max).

Parameter at peak lactation	Standard (n=6)	Gel (n=6)	Bowl (n=6)	Control (n=6)
Total protein (g/L)	54 ± 4 (58-69)	55 ± 4 (50-61)	55 ± 6 (47-63)	63 ± 8 (57-78)
Hemoglobin (g/L)	158 ± 11 (145-172)	160 ± 10 (145-171)	155 ± 14 (137-171)	162 ± 10 (145-173)
Hematocrit (%)	48 ± 4 (44-53)	49 ± 3 (44-52)	47 ± 4 (42-52)	59 ± 3 (44-53)
Serum osmolality (mOsmol/kg)	325 ± 14 (308-348)	329 ± 13 (313-343)	328 ± 8.7 (319-341)	333 ± 8.0 (322-346)
Blood urea nitrogen, BUN (mg/L)	380 ± 51 (320-440)	326 ±101 (170-410)	254 ± 86 (190-400)	383 ± 103 (320-440)
Magnesium (mmol/L)	1.1 ± 0.9 (1.1-1.2)	1.1 ± 0.8 (1.0-1.2)	1.1 ± 0.1 (0.9-1.3)	1.1 ± 0.1 (1.0-1.2)

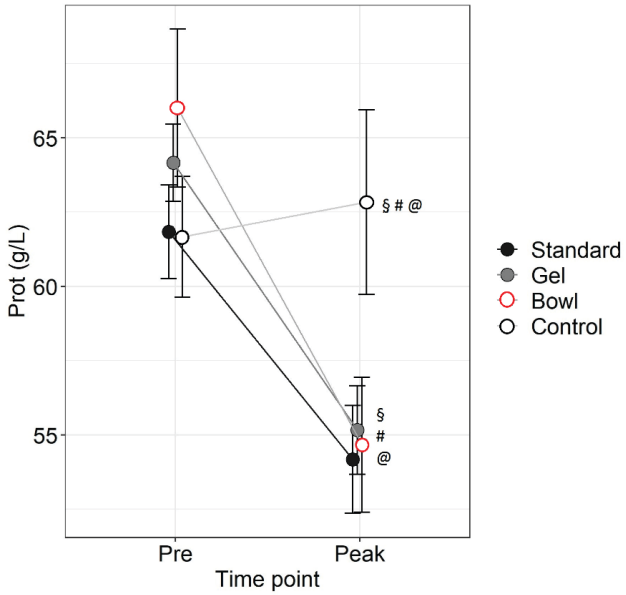

Figure 2. Total protein levels before (Pre) and at peak lactation in RjOrl:SWISS and C57BL/6JRj mice with different types of watering: standard water bottle (Standard), bottle plus water gel (Gel) or bottle plus water bowl (Bowl). Non-lactating mice were used as control. N=6 per group. Data are means ± SEM (§§ = p<0.05, @@, ## = p<0.01, two-way RM ANOVA, *post hoc* test LSD).

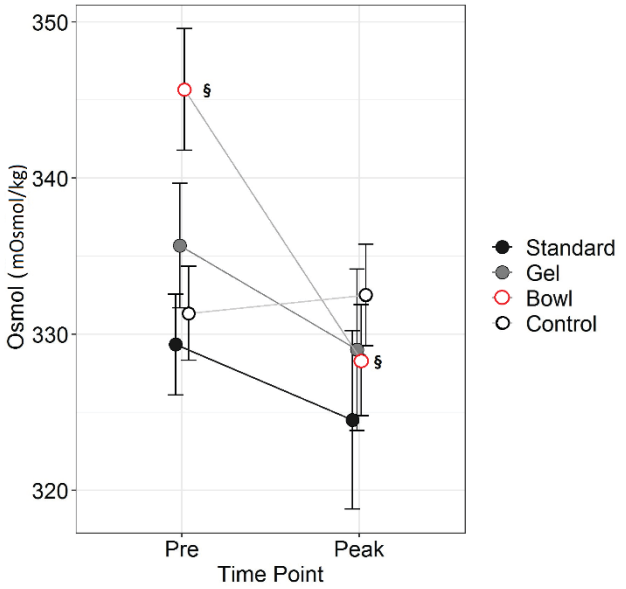

Figure 3. Osmolality before (Pre) and at peak lactation in RjOrl:SWISS and C57BL/6JRj mice with different types of watering: standard water bottle (Standard), bottle plus water gel (Gel) or bottle plus water bowl (Bowl). Non-lactating mice were used as control. N=6 per group. Data are means ± SEM (§§ = p<0.01, two-way RM ANOVA, *post hoc* test LSD).

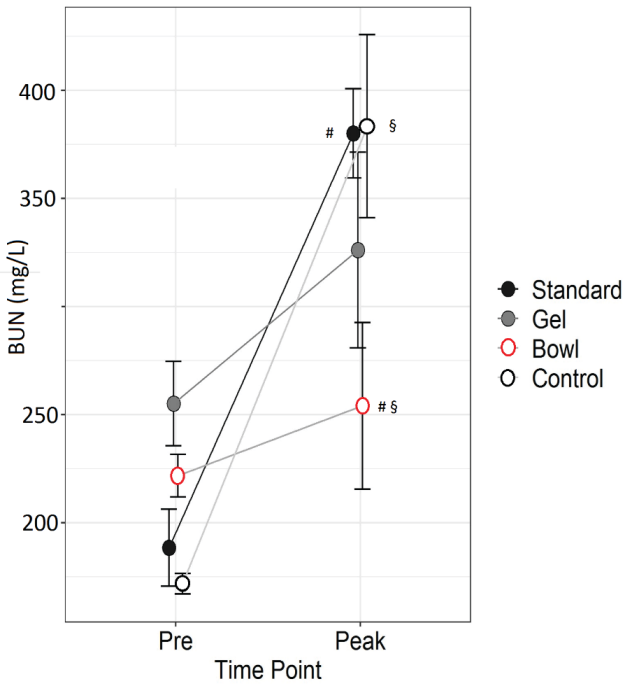

Figure 4. Blood Urea Nitrogen (BUN) before (Pre) and at peak lactation in RjOrl:SWISS and C57BL/6JRj mice with different types of watering: standard water bottle (Standard), bottle plus water gel (Gel) or bottle plus water bowl (Bowl). Non-lactating mice were used as control. N=6 per group. Data are means ± SEM (§§, ## = p<0.01, two-way RM ANOVA, *post hoc* test LSD).

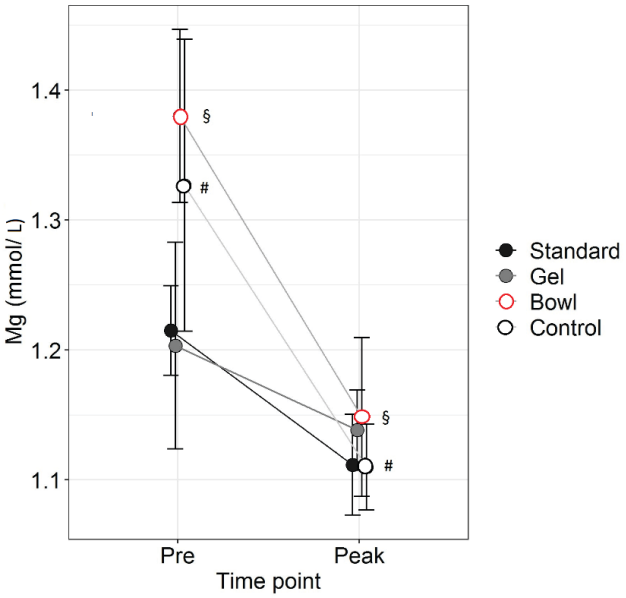

Figure 5. Magnesium before (Pre) and at peak lactation in RjOrl:SWISS and C57BL/6JRj mice with different types of watering: standard water bottle (Standard), bottle plus water gel (Gel) or bottle plus water bowl (Bowl). Non-lactating mice were used as control. N=6 per group. Data are means ± SEM (§§, ## = p<0.01, two-way RM ANOVA, *post hoc* test LSD).

also had an effect on consumption (p<0.001). There was no interaction between strain and group (p=0.3). Mice in group Bowl drank less from the bottle than Gel and Standard (p<0.001). Gel and Standard did not differ (p=0.3).

When bottle water consumption was related to body weight of the female and the litter, the consumption was instead higher in B6 than in SWISS mice (p=0.002), and there was a main effect of group (p<0.001) as well as an interaction between strain and group (p=0.02, two-way ANOVA, non-transformed data). In both SWISS and B6 mice, water consumption was lower in Bowl than Standard (p=0.004 and p< 0.001, respectively) and was lower than Gel (p=0.01 and p<0.001, respectively).

A higher number of pups per female were weaned from SWISS [13 (3-17), n=9)] than B6 mice [5 (3-12), n=9, p< 0.001, Mann-Whitney test]. There was no difference in the number of pups weaned between groups (Standard 10, Gel 7.5, Bowl 11.5, p=1.0, Kruskal Wallis test). SWISS pups weighed more [21.5 (14.3-28.5g)] than B6 pups [12.4 (10.6-16.7g), p<0.001, Mann-Whitney test]. Average pup weight, at four weeks of age, was higher in Bowl (19

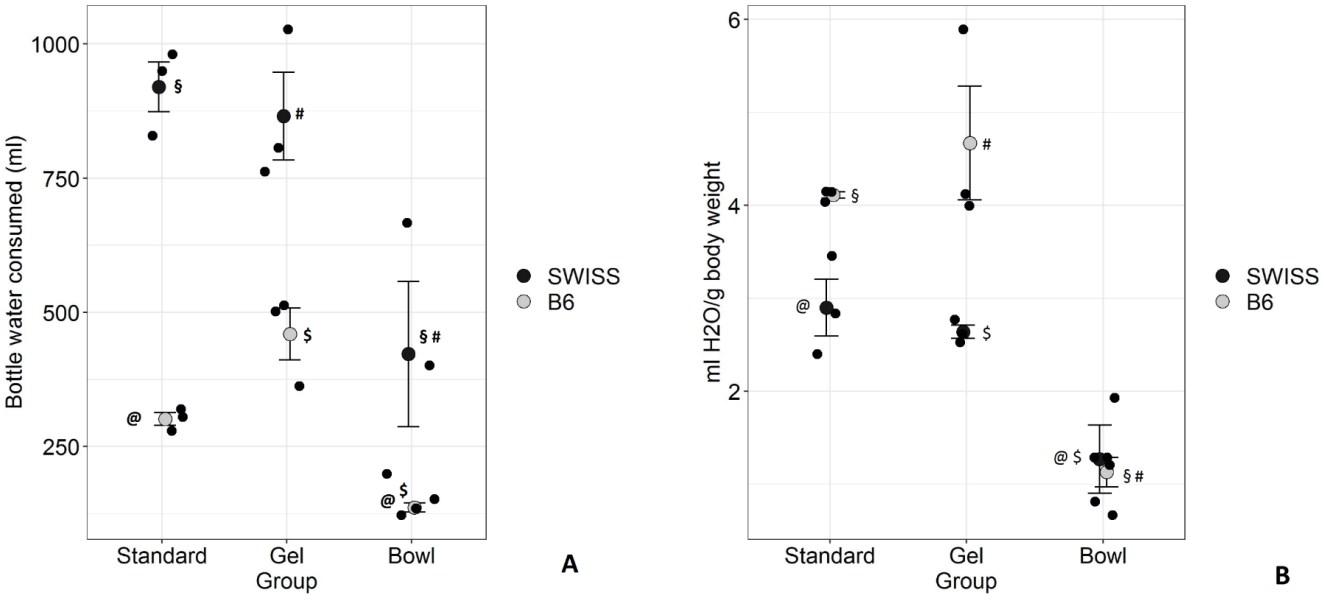

Figure. 6. Bottle water consumption during lactation in absolute volume (A) and relative to body weight of female and litter at weaning (B) in two strains of mice: RjOrl:SWISS (n=9) and C57BL/6JRj (n=9). Standard= water bottle, Gel= water bottle plus a water gel sachet, Bowl= water bottle plus a water bowl. **A:** SWISS mice consumed more bottle water than B6 across groups (p<0.001) and mice in Bowl consumed less than Gel and Standard in both SWISS (§§, ## = p<0.05) and B6 mice (@@,$$= p < 0.01). **B:** Water consumption/g was higher in B6 than SWISS mice in Standard and Gel (p<0.05 and p<0.001, respectively) and Bowl was lower than Standard and Gel in both SWISS (@@, $$ = p<0.01) and B6 (§§, ## = p< 0.001, two-way ANOVA; *post hoc* LSD). Individual data and means ± SEM.

± 5g) and Gel (18 ± 6g) compared with Standard (14.5 ± 4g, p=0.008 and 0.02, respectively, two-way ANOVA).

Discussion

The present study was undertaken to establish if female mice are dehydrated during peak lactation, when water is provided in bottles, and if additional provision of water in a bowl or as gel has any effect on hydration parameters. Two strains of female mice were examined during their second lactation, as female mice usually have smaller first litters (Brown et al. 1999).

According to the measured blood, plasma and serum parameters, neither SWISS nor B6 mice were dehydrated before mating, or during peak lactation. However, adding a water bowl to lactating mice led to a marked reduction (54%) of bottle water consumption. The differences were most pronounced if water consumption was related to body weight. Mice showed no interest in ingesting the water gel, but rather manipulated it and spread it around the cage, which prevented the measurement of ingested amount. The bottle water consumption was very similar in group Gel and Standard, and in both cases was higher than in Bowl.

Lactation requires a very high energy intake for mice. It has been estimated that in relation to body weight, a mouse produces about 16 times more milk per day than a cow (Hanwell 1977). At peak lactation, a four to five-fold increase in feed intake is required, and mice need to drink over 60 % of their body weight (Murai et al. 2013). Even if drinking from a bottle may be a restriction, the data shows that female mice keep a physiological water balance during peak lactation. In a study where non-lactating mice were exposed to chronic water restrictions of up to 50% of the daily ration for 8 days, none of the hydration related variables (Osmol, Htc, TP) showed a significant change from controls, even though mice were clearly thirsty and lost 10 % of their body weight (Bekkevold et al. 2013) . This indicates that mice are equipped to deal with water restriction, which is likely a physiological adaption for survival inherited from their wild ancestors. Lactating mice that were fed a high potassium chloride supplement during lactation increased their water intake by 100%, which indicates a capacity to substantially increase water intake, but with the cost of a 15 % reduction in body weight of the females and 15 % less body weight gain of the pups (Murai et al. 2013).

It was not possible to measure the water intake from the bowl, since evaporation, splashing and contamination with bedding occurred. Nevertheless, the reduced intake from the bottle indicates that mice prefer to drink water from a bowl, which is in line with data from a similar pilot study in fancy mice, in which the decreased bottle water intake was even larger (67 %). Pet rabbits have been shown to prefer drinking, and to drink faster and more, from open dishes compared with nipple drinkers (Tschudin et al. 2011). This has led to the recommendation to provide rabbits with water in open dishes, since reduced water intake is linked to urinary tract disease. Horses show a strong preference for drinking water from a bucket compared with an automatic water bowl, even when the automatic flow rate approximates to their normal drinking speed (Nyman and Dahlborn 2001).

There was further evidence from measured blood parameters that lactating mice with access to a water bowl drank more; BUN was 34 % lower in Bowl at peak lactation compared with both Standard and Control, and only in Bowl was plasma osmolality lower (5 %) at peak lactation compared with Pre.

In a study in Wistar rats, the changes in plasma osmotic pressure during lactation were examined and it showed that total protein, Htc and plasma Osmol were reduced during lactation, in comparison to dams that had the pups removed at birth (Suzuki et al. 1993). In the present study, no effect on Htc was seen and the Osmol did not differ from controls, although in group Bowl, it was lower at Peak than Pre. The decrease of plasma Osmol in the rats was explained by hydro-dilution, which supports the suggestion that the mice in the present study drank more with a bowl present and thereby diluted the blood.

Total plasma protein was lower in all lactating mice compared with control, on average the total plasma protein was 13 % lower during lactation. This has been seen in several domestic species where plasma proteins, and especially albumin, decrease during lactation (Eckersall 2008) and in one study with CD1 mice the albumin concentration in serum was shown to be reduced by 50 % or more during peak lactation (Monks and Neville 2004). Lactating female mice produce high protein content milk as well as an increase in protein synthesis in the mammary glands during lactation (Millican et al. 1987).

Magnesium levels did not differ between groups at peak lactation, although mice in group Bowl had lower levels compared with Pre. We included Mg in the analyses to investigate whether lactating mice had higher levels of Mg during peak lactation, because a previous study showed that mice which died from

paralytic ileus during peak lactation had increased Mg serum, with levels up to 6.5 mmol/L (Tjäder et al. 2013). Hypermagnesemia is an uncommon finding in mammals, since excess magnesium is normally excreted by the kidneys, but has been shown to cause paralytic ileus in humans (Golzarian et al. 1994; Izdes et al. 2008). Restricted water intake during lactation could possibly lead to an accumulation of magnesium, which is absorbed by passive diffusion, and cause a reduction of peristalsis of the gut (Glozarian et al. 1994). Mouse feed has been shown to contain up to 7 times the recommended amount of magnesium for lactating females (Wise and Gilburt 1981; National Research Council 1995)

Four weeks old pups weighed more in group Bowl (mean weight: Bowl 19 g; Gel 18 g; Standard 15 g) and pup weight was related to number of pups in the litter. The median number of pups was however higher (non significant) in group Bowl than Standard or Gel (11.5, 10 and 7.5, respectively). Pups start eating solid food at day 16-17 *postpartum* (Pritchett and Taft 2007) and since water and feed intake are closely linked, easy access to water may have increased the solid feed intake in both pups and females. It could also indicate that the females with the water bowl produced more milk.

The present study was initially planned with twice the number of mice, divided into two blocks. The second block was never performed because the results showed that none of the 18 lactating mice in the first block showed evidence of dehydration. It was therefore decided not to carry out the second block, in accordance with the 3Rs. Since most parameters were not affected by strain, the data from both strains could be pooled, which made the groups large enough to establish clear effects of water supplementation. There is a high number of influencing factors for many clinical chemistry plasma analytes in mice which makes it difficult to define universally valid normal values (Otto et al. 2016). The presented data are however mostly in the range of published levels for mice. Factors such as diet, housing, blood sampling procedure, pre-analytical handling of samples, equipment and methods used for analyses are known to affect the results.

In summary, the study shows that mice do not suffer from dehydration during peak lactation. Mice however prefer to drink, and seem to drink more water, from a bowl than from a bottle.

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Conflict of interest

The authors declared no potential conflicts of interest.

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