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Influence of cross-fostering on preference for calcium chloride in C57BL/6J and PWK/PhJ mice

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Abstract

We investigated whether maternal influences during the suckling period alter the avidity for calcium, using as models mice from the calcium-preferring PWK/PhJ strain and the calcium-avoiding C57BL/6J strain. We found that milk collected from PWK/PhJ dams had higher calcium concentrations than did milk collected from C57BL/6J dams. Despite this, cross-strain fostering had no effect on adult calcium preferences relative to mice of the same strain that were within-strain fostered or not fostered. Our results indicate that calcium avoidance by C57BL/6J mice and acceptance by PWK/PhJ mice is unaffected by maternal environment during the suckling period.

Keywords

calcium; mineral appetite; maternal; lactation; taste; foster

1. Introduction

Calcium is an essential nutrient involved in many physiological processes. Extracellular calcium concentrations are tightly regulated at ~1 mM despite wide fluctuations in calcium intake from food [1]. Calcium salts in concentrations around those of extracellular fluid are preferred to water by humans [2] and rodents [3] but at higher concentrations (i.e., > 5 – 300 mM) they are considered unpalatable [4-6] and most strains of rats and mice avoid them [7,8]. There are exceptions, however. Mice of some inbred strains readily ingest concentrated calcium solutions even when they are fed calcium-replete food. A notable example is the PWK/PhJ (PWK) strain, which has the highest preferences for several concentrations of calcium chloride (CaCl₂) and calcium lactate out of 40 strains surveyed [9]. This avidity for calcium is accounted for by two genetic loci. One is the *Tas1r3* gene, which also encodes a subunit of the sweet and umami taste receptors [10]; the other is a locus on chromosome 16 that has not been definitively identified, although a strong candidate is *Casr*, the calcium-sensing receptor, which is present in taste buds and has been implicated in taste perception [11-15].

In rats and mice, the acceptance of calcium has a strong innate component [16-18] and is highly heritable [9]. However, there are reasons to suspect that calcium consumption is also shaped by environmental and maternal influences in early life. First, maternal environment

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can interact with the existing genotype of the offspring and induce epigenetic modifications that result in enhanced or reduced expression of complex traits such as body weight, food intake and insulin sensitivity. In some cases maternal environment can even overshadow genetic factors [19-21]. Second, the gustatory system in rodents is immature at birth. Taste buds continue to develop during the first postnatal weeks, the afferent terminal field of the major gustatory nerves including the chorda tympani nerve (which carries calcium-specific nerve fibers and is involved in regulation of calcium consumption [22-24]) undergoes reorganization, and responses of the nerves to gustatory stimuli change during postnatal development [25-28]. The development of the mechanisms that mediate the regulation of calcium intake parallels the ontogenetic changes within the gustatory system: rat pups start to respond to calcium deficiency with increased calcium intake on postnatal day 4 and this becomes progressively more specific throughout the rest of the suckling period [29].

Thus, the question arises of whether the avidity for calcium can be modified during the first weeks of life when a mouse pup's diet constitutes its mother's milk, which contains calcium, calcium-regulatory hormones and other factors that have the potential to influence calcium preference. Milk calcium content is a very stable factor: it does not vary much during the lactation period and is unaffected by the mother's dietary calcium content during lactation [30,31]. However alterations of the genetic background that affect calcium metabolism have an effect on calcium milk content that, in turn, affects the calcium status of the offspring [32].

Here we assessed the influence of maternal environment during the suckling period on calcium acceptance in mice of a calcium-avoiding strain (C57BL/6J; B6) and the calcium-preferring PWK strain [9]. First, we measured milk calcium concentrations and found that PWK dams provide their pups with milk that has a ~ 40% higher calcium concentration than do B6 dams. Then, to manipulate the maternal environment, newborn pups were reciprocally cross-fostered between dams of the opposing strain until weaning; control group pups stayed with their biological mother or, to control for the influence of the fostering procedure, some pups of each strain were also in-fostered to mothers of the same strain. When fostered and control mice reached 8 weeks of age they received two-bottle choice tests between 25 mM CaCl_2 and deionized water.

2. Methods

The experiment protocol was approved by the Monell Chemical Senses Center Institutional Animal Care and Use Committee.

2.1. Animals and housing conditions

Six B6 and 4 PWK lactating dams served as milk donors for calcium measurements. For the fostering experiment, 13 B6 and 12 PWK males and 13 B6 and 12 PWK (8 - 14 weeks old) females were used as parents to produce 33 litters consisting of 50 B6 and 58 PWK progeny (see below). The mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA; catalogue no., B6 = 000664, PWK = 003715) or were bred in our facility.

All mice were maintained in a vivarium at 23°C with a 12:12 h light/dark cycle (lights off at 19:00 h). They were housed in plastic "tub"-style cages (26.5 cm × 17 cm × 12 cm) with stainless steel wire lids and wood shaving bedding. When not being tested, the mice had free access to deionized water provided in 300-mL glass bottles with stainless-steel spouts. AIN-76A purified pelleted diet (#100000, Dyets, Bethlehem, PA; 130 mmol calcium/kg diet) was available *ad libitum*.

2.2. Milk collection and total calcium measurements

Milk from lactating dams was collected on day 13-16 postpartum. Dams were separated from their litters ~ 2.5 h prior to milking. Each dam was deeply anesthetized with 1% isoflurane and it then received 0.1 ml (2 IU) of oxytocin by intraperitoneal injection. To facilitate milk release, gentle pressure was applied to the base of the teats. Expelled milk was collected by aspiration. Milk yield varied from ~10 to 100 μ L per mouse. All samples were stored at -20° C. Milk samples were diluted 1:40 with water and then total calcium content was assessed using a colorimetric assay based on the interaction of calcium with α -cresophthalein (BioVision, Mountain View, CA).

2.3. Breeding and cross-fostering

To produce offspring, each adult female was housed with a male of the same strain. When the female showed signs of being pregnant (i.e., had a notable increase in body weight and an enlarged abdomen) the male was removed. The resulting pups were fostered on postnatal Day 2 and weaned on postnatal Day 21 - 23. We randomly assigned half-litters to the following experimental groups: Pups raised by their own mothers (i.e., not fostered; referred to as B6-0 and PWK-0), pups fostered to a mother of the same strain (i.e., in-strain fostered; B6-IF and PWK-IF), and pups fostered to a mother of the other strain (i.e., between-strain cross-fostered; B6-CF and PWK-CF). To accomplish this, litters were split and reciprocally fostered between litters of comparable size (i.e., ± 1 pup) born no more than one day apart. So, half the pups from a litter were fostered and the other half stayed with their mother but had pups of another mother (of the same or opposite strain) as their littermates. If there was an uneven number of pups in the litter ($n \pm 1$)/2 pups were fostered depending on the size of the recipient litter (e.g., 3 pups were fostered between litters of 6 and 7 pups, 4 pups between litters of 7 and 8 pups); the rest of the litter stayed with their mother. This maintained the initial size of the litter in PWK dams as they reacted with a higher rate of infanticide to any changes related to the fostering procedure. For the fostering procedure, the dam was removed to a clean separate cage for 1 h, while the foster and biological pups were mixed together and carefully rubbed against nesting material. Group sizes, litter sizes, the number of litters, the number of males and females within the each group and mice body weights were recorded and are provided in Table 1.

2.4. Behavioral testing

After weaning, mice were housed with littermates of the same sex in groups of 3 - 4 mice until they were 7 - 9 weeks old (average, 57.0 ± 0.4 days). They were then housed individually for 8 - 10 days. Body weights were recorded to the nearest 0.1 g using a top-loading scale and the mice began two-bottle choice tests. First, the intakes from two tubes, each containing deionized water, were recorded for 2 days. Then, the mice were given a choice between deionized water and 25 mM CaCl_2 solution (Sigma-Aldrich, St. Louis, MO; dissolved in deionized water) and their 24-h intakes from each tube were recorded for 4 consecutive days. The testing apparatus and procedures are described in detail elsewhere [33,34]. Briefly, the regular drinking bottle was replaced with two graduated drinking tubes that allowed water and solution intake to be recorded volumetrically to the nearest 0.1 mL. On the first day of each test, the solution was presented on the left. Every 24 h, the volume of remaining fluid was recorded and the positions of the solution and water tubes were switched to account for side preferences.

2.5. Data analysis

We used a Mann-Whitney U-test to compare the calcium concentrations of milk from B6 and PWK dams. We consider a nonparametric test appropriate for this because there was

insufficient information available to ascertain the distribution of calcium milk concentrations.

Preferences for CaCl_2 were calculated by dividing the average 4-day intake from the solution tube by the average total 4-day intake from both tubes (average 4-day intake from solution tube + average 4-day intake from water tube). Side preferences were calculated from the 2-day test when mice were presented with deionized water only; average intake from the tube on the left was divided by the average total intake from both tubes. Preference for CaCl_2 , total water intake (derived from the 2-day test with deionized water only), side preferences and body weight were analyzed using analyses of variance with categorical factors of Sex, Strain and Neonatal Treatment (0, CF, IF). Differences in litter size between the groups were assessed using a two-way analysis of variance with factors of Strain and Neonatal Treatment. When statistically significant effects were revealed ($p < 0.05$) post-hoc Tukey's Honestly Significant Difference tests were used to identify homogenous groups. All statistical analyses were conducted using the Statistica software package [35].

3. Results

Total calcium concentrations were significantly higher in the milk samples acquired from PWK dams compared with B6 dams (Mann-Whitney $U = 0.0$, $p = 0.014$; Fig.1).

All B6 mice had a significantly lower preference for CaCl_2 than did PWK mice irrespective of their fostering history (Strain: $F_{1,100} = 329.7$, $p < 0.001$; Fig.2). Fostering had no influence on CaCl_2 preference of either B6 or PWK mice.

Mice of the B6 strain were heavier and had higher total fluid intakes than did the mice of the PWK strain (body weight: Strain: $F_{1,100} = 340.0$, $p < 0.001$; total fluid intake: Strain: $F_{1,100} = 130.0$, $p < 0.001$) but there were no differences in body weight or total water intake between the foster groups of the same strain. B6 and PWK males were significantly heavier than B6 and PWK females respectively (Sex: $F_{1,100} = 49.5$; $p < 0.001$, Sex $\times$ Strain: $F_{1,100} = 8.9$, $p = 0.0014$, post-hoc: $p < 0.001$ for B6, $p = 0.02$ for PWK) (Table 1). Sex did not influence water intake or calcium preference. There were no differences in side preferences between the groups. There were differences in litter size among experimental groups (Strain $\times$ Neonatal Treatment: $F_{2,106} = 5.5$, $p = 0.005$, post-hoc: $p < 0.01$ for B6-0 vs PWK-0, $p < 0.05$ for all other significant differences in litter size; please see Table 1 for detail).

Discussion

We investigated whether maternal environment influences the avidity for calcium in mice that are genetically predisposed to avoid (B6) or prefer (PWK) calcium. We found that despite large differences in milk calcium concentrations, reciprocal cross-fostering of pups between litters of B6 and PWK mice during the suckling period had no effect on their adult CaCl_2 preferences. Thus, the PWK strain's avidity for calcium is not formed during the suckling period and does not depend on milk calcium content; similarly, an avidity for calcium could not be induced in B6 mice by exposing them during the suckling period to increased milk calcium concentrations or to the rearing practices of PWK mice.

The high levels of calcium in milk from PWK dams may reflect a general shift in calcium homeostasis of the PWK strain towards reduced calcium retention. It is consistent with the relatively low plasma ionized calcium concentrations and low bone mineral density of this strain[9]. Irrespective of the physiological mechanism, our observation that B6 and PWK milk differs markedly in calcium concentration suggests that the two strains are normally supplied with different amounts of calcium during suckling. However, some caution in interpretation is required because we sampled milk at only one time (13-16 days postpartum)

and did not assess the volume of milk the pups ingested. Suckling pups react to disturbances in their calcium homeostasis with changes in ingestive behavior [29] so it may be that pups titrate concentration and volume to regulate calcium intake.

It is also possible that maternal environment during the lactation period affects the avidity for calcium but the effect is transient and not preserved into adulthood. In this respect, we note that alterations in gustatory nerve responses to sodium chloride are apparent in pups born to sodium-restricted mothers but can be completely reversed if pups are transferred to a sodium-replete diet in the postnatal period [36,37]. If avidity for calcium is regulated similarly then exposure to calcium-replete diet may have reversed any transient alterations in gustatory functions caused by early experience. However, such a reversible influence cannot be responsible for shaping the high preferences for calcium of the PWK strain.

Our results demonstrate that the avoidance of calcium shown by the B6 strain, and the preference for calcium shown by the PWK strain, do not require nursing by the mother of the same strain for the full expression of this phenotype. However, it is possible that maternal influences play some role during the gestation period. Maternal dietary sodium restriction affects gustatory responses only if it is implemented before embryonic day 8 [38]. Thus it would not be surprising if a sensitive period also exists in the formation of calcium gustatory responses.

Conclusion

This is the first study to investigate maternal influences on calcium preferences in inbred mouse strains differing in preferences for calcium. Lactating dams of the PWK strain have higher total calcium levels in their milk than do B6 dams. Changing maternal environment during the suckling period by cross-fostering between the strains had no effect on preferences for calcium in adulthood. We conclude that the PWK strain's high avidity for calcium and the B6 strain's avoidance of calcium are either dependent solely on genetic factors or, if dependent on maternal environment, are shaped prenatally.

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Highlights

- We investigated whether early maternal experience influences calcium preference
- Calcium-liking PWK/PhJ and calcium-avoiding C57BL/6J mice were compared
- Milk of PWK dams had higher calcium concentrations than did milk of B6 dams
- Cross-fostering had no effect on either strain's adult calcium preferences
- The avidity for calcium is not formed during the suckling period

Figure 1.
Total calcium concentrations in the milk of C57BL/6J (B6) and PWK/PhJ (PWK) dams.
Data presented as mean $\pm$ SEM, * $p = 0.014$.

Figure 2. Mean $\pm$ SEM preference scores for 25 mM CaCl₂ by C57BL/6J (B6) and PWK/PhJ (PWK) mice that were cross-fostered (CF), in-fostered (IF) or not fostered (0). Groups sharing the same letter did not differ significantly.

Table 1

Group sizes, body weight, number of litters and litter size of C57BL/6J (B6) and PWK/PhJ (PWK) mice raised in different maternal environments.

Group	No. of litters	Pups per litter	Females		Males	
			n	body weight, g	n	body weight, g
B6-0	6	7.1 ± 0.3 ^c	10	18.8 ± 0.5	10	23.6 ± 0.5
B6-CF	6	5.5 ± 0.4 ^a	7	18.2 ± 0.8	6	22.0 ± 0.7
B6-IF	4	7.1 ± 0.4 ^{bc}	8	18.8 ± 0.6	9	22.3 ± 0.6
PWK-0	6	5.3 ± 0.3 ^a	15	12.5 ± 0.4	10	13.6 ± 0.6
PWK-CF	7	5.8 ± 0.3 ^{ab}	11	12.8 ± 0.5	8	14.0 ± 0.6
PWK-IF	4	7.1 ± 0.4 ^{bc}	7	12.4 ± 0.7	7	13.8 ± 0.7

Notes: B6 = C57BL/6J, PWK = PWK/PhJ; 0 = not fostered, CF = cross-fostered (i.e., to a mother of other strain); IF = in-fostered (i.e., to another mother of same strain). Values are means ± SEM; n = pups surviving at weaning. Groups sharing the same letter did not differ significantly. Body weight refers to weight at beginning of choice test, when mice were adult. B6 mice were significantly heavier than PWK mice and males were significantly heavier than females. There were no significant differences in body weight within a strain and sex.