

Reproductive and neurobehavioral outcome of drinking purified water under magnesium deficiency in the rat's diet

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Abstract

Taking magnesium deficient diet and drinking soft water (including purified water, essentially mineral free) are common consumed in the world. The present study was conducted to assess the potential combined influence of maternal drinking purified water and taking magnesium deficient diet on postnatal development and behavior in the offspring of exposed rats. Sprague–Dawley (SD) rat were assigned to four groups: group 1 fed with control diet (Mg^{2+} 0.4 g/kg) and control water (Mg^{2+} 12.7 mg/L), group 2 fed with control diet and purified water (Mg^{2+} 0.015 mg/L), group 3 fed with magnesium deficient diet (Mg^{2+} 0.2 g/kg) and control water, group 4 fed with magnesium deficient diet and purified water from 5 weeks of age of the F0 generation to 3 weeks of the F1 generation, respectively. Reproductive and neurobehavioral parameters were measured. Maternal body weights significantly decreased during treatment (before mating) and lactation periods in the group fed with magnesium deficient diet and purified water. There were no significant differences of the reproductive outcome in the groups. Offspring's body weight, development of reflexes significantly reduced in the group 4. Although it was no significant difference in the four groups, the data showed a trend toward a decreased risk for offspring body weight, neurobehavioral development as follows: group 1 > group 2 > group 3 > group 4. Therefore, purified water cannot obviously affect the reproductive outcome when magnesium is sufficient or half of the estimated average requirement (EAR) in the diet. However, drinking purified water can decrease maternal magnesium level slightly, induce offspring's development retardation, especially when the magnesium deficiency in the diet. Furthermore, magnesium deficiency in the diet (half of the EAR) can produce growth delay and reflex development retardation in F1-offspring. Therefore, drinking purified water should be carefully considered, especially for susceptible population.

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1. Introduction

Good health begins with good water. It is reported that not only things like parasites, disinfection by-products and other contaminants in the drinking water could affect peo-

ple's health; the constituents in the water also affect people's health, especially the natural water constituents. Calcium and magnesium are major constituents of natural water have been extensively studied for years.

Both of these elements are essential for the human body. Although foods are the major source of mineral nutrients, a series studies in industrialized countries reported that people intake of calcium (Ca) and magnesium (Mg) were lower than the recommended dietary allowances by consuming usual diet. Most American took less Ca and Mg than recommended and some subjects took less than 80% of recommended dietary allowance for Mg (Marx and Neutra, 1997; Ford and Mokdad, 2003). Similar data were

Abbreviations: EAR, estimated average requirement; GD, gestation day; PND, postnatal day; Ld, lactation day; NRC, National Research Council; SD, Sprague–Dawley; COD, chemical oxygen demand; TDS, total dissolved solids; IHD, ischemic heart disease; Ca, calcium; Mg, magnesium; ANOVA, analysis of variance.

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observed in other European countries, such as Czech, Northern Germany (Ruprich et al., 2001; Schimatschek, 2003). Another study reported that the intakes of calcium, zinc, magnesium, potassium and other essential minerals were insufficient, and which is a traditional problem in Chinese diet (Chen and Gao, 1993). Therefore, the supplement from drinking water is crucial.

Epidemiological studies showed that drinking water is also an important source of essential elements such as Ca and Mg (Widdowson, 1944). Calcium and magnesium are mainly present as simple ions Ca^{2+} and Mg^{2+} in water, and it has been suggested that the water magnesium and calcium is more bioavailable to a higher content (from 40% to 60%) than the diet magnesium and calcium (Sabatier et al., 2002). Only a small proportion (less than 5–10%) of the daily intake of magnesium comes from drinking water but Mg absorbability from water is 30% higher compared to dietary magnesium (Marx and Neutra, 1997). A research in a French adult population indicated that mineral water may contribute to one fourth of the total daily calcium intake and contributed 6–17% of total daily magnesium intake (Galan et al., 2002). Additionally, a Swedish study including an oral loading test with magnesium suggested that the body magnesium level can be affected significantly by magnesium in the drinking water (Rubenowitz et al., 1998).

The content of calcium and magnesium determine the hardness of drinking water. Researchers have reported an inverse correlation between water hardness and mortality from cardiovascular disease (Durlach et al., 1985), a significant link between water hardness and cerebrovascular disease, ischemic heart disease (IHD) (Sauvant and Pepin, 2002). They also observed a correlation between soft water or low mineral drinking water and hypertension, coronary heart disease, pregnancy complications and several complications in infants (Chiu et al., 2005; Kozisek, 2004).

However, drinking soft water is common in most parts of Asia, Latin America, Africa, America, and Europe. The levels of calcium, magnesium and zinc in soft water are usually low. In a survey in Sina Net in 2006 indicated that it is almost 20.47% people (4606 subject take part in the survey) drink purified water (essentially mineral-free water). Bottled water is the fastest growing drink choice in the worlds because of its taste, and most of them are soft water such as distilled water, purified water, and sterile water. Carbonates water, soda water, seltzer water, sparkling water, tonic water are also considered soft drinks. Bottled waters in North America have low mineral contents. Consuming a magnesium deficient diet and/or drinking soft water prevail in the world, the health effect of which has attracted some researchers. A study in our department suggest even the micronutrients is sufficient in the food, drinking purified water for a long time could result in the deficiency of magnesium in females (Shu et al., 2001). Magnesium deficiency may induce hypertension, placental abruption, still birth and low birth weight (Pathak and Kapil, 2004). And it is reported that use of soft water, especially those that are low in calcium and magnesium, has

also been associated with very low birth weight, suggesting that drinking water with low mineral content may affect the fetal growth (Yang et al., 2002a,b).

In order to gain a better understanding of the effect of the soft drinking water on the offspring, we investigate the relationship between purified drinking water (purified water is extremely soft water) and maternal reproductive performance and offspring physical development and neurobehavioral function under maternal micronutrient sufficient diet or magnesium deficient diet.

2. Materials and methods

2.1. Animals and materials

The experimental diets were prepared bimonthly (twice) in our laboratory, and it is based on the AIN-93G Purified Rodent Diet (DYETS #110700), except that choline chloride replaces the choline bitartrate, and the mineral mix differed only with respect to magnesium content.

Control diet: all the micronutrients are sufficient for good health, according to AIN-93M-GX Mineral Mix (DYETS #210050) except Mg^{2+} content is 0.4 g/kg, according to estimated average requirement (EAR, from National Research Council, NRC, 1978) for rats.

Magnesium deficient-diet: Mg^{2+} content is approx. 0.2 g/kg, a half of the estimated average requirement (EAR, from NRC) for rats.

Control drinking water: total dissolved solids (TDS) 175 mg/L, conductivity 350 $\mu\text{S}/\text{cm}$, chemical oxygen demand (COD) under detection, Mg^{2+} 12.7 mg/L.

Purified drinking water: TDS 1.16 mg/L, conductivity 2.28 $\mu\text{S}/\text{cm}$, COD under detection, Mg^{2+} 0.015 mg/L.

Weaning SD rats (68 females, 50–75 g; 34 males, 50–75 g) were obtained from the Laboratory Animal Center of this university (license number: SCXK-2002-008). They were acclimated for 1 week before being placed on the study. The animals were assigned to the four groups by the randomization method, group 1 fed with control diet and control water, group 2 fed with control diet and purified water, group 3 fed with magnesium deficient diet and control water, group 4 fed with magnesium deficient diet and purified water throughout the entire study, respectively. All the animals had free access to diet and water.

They were housed individually in polycarbonate solid-floored cages with wood flakes, and kept on a 12 h light/12 h dark cycle in a temperature-controlled room maintained at $25 \pm 1^\circ\text{C}$ with relative humidity of $50 \pm 5\%$.

2.2. F0 reproductive procedure

Diet and water were given from 5 weeks of age of the F0 generation to 3 weeks of age of the F1 generation. All offspring were examined for behavior development during the lactation period.

The animals from the F0 generation were 5 weeks of age at the start of the study. The animals were weighed individually on experimental day 0, 7, 14, 21, 28, and 35 during the preconception period. At 9 weeks of age (the rats were sexual mature), two females were paired with one male (2:1) from the same treatment group for a period of 14 days until mating was confirmed by observation of a copulatory plug or the presence of sperm in a vaginal rinse. The day that mating was confirmed was recorded as gestation day (GD) 0. The mated females were weighed on days 0, 6, 12 and 18 of gestation and 0, 4, 7, 14 and 21 of lactation. The day of birth was identified as postnatal day 0 (PND0). Postnatal survival was monitored daily before weaning (day 21).

2.3. Offspring studies

All the pregnant rats were allowed to give birth (F1-generation) and nurture their offspring normally. On the day parturition was initiated

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