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INFLUENCES OF ALKALINE IONIZED WATER ON MILK ELECTROLYTE CONCENTRATIONS IN MATERNAL RATS

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ABSTRACT — We previously reported that body weight on day 14 after birth in male offspring of rats given alkaline ionized water (AKW) was significantly heavier than that in offspring of rats given tap water (TPW), but no significant difference was noted in milk yield and in suckled milk volume between the two groups. Additionally, the offspring in the AKW group and TPW group were given AKW and TPW, respectively, at weaning, and unexpectedly, the necrotic foci in the cardiac muscle were observed at the 15-week-old age in the AKW group, but not in the TPW group. The present study was designed to clarify the factors which are involved in that unusual increase of body weight and occurrence of cardiac necrosis. Eight dams in each group were given AKW or TPW (control) from day 0 of gestation to day 14 of lactation. The milk samples were collected on day 14 of lactation and analyzed for concentrations of calcium (Ca), sodium (Na), potassium (K), magnesium (Mg) and chloride (Cl). The AKW and TPW were also analyzed. Ca, Na and K levels in milk were significantly higher in the AKW group compared to the TPW group. No significant difference was noted in the Mg and Cl levels between the two groups. These data suggested that the Ca cation of AKW enriched the Ca concentration of the milk and accelerated the postnatal growth of the offspring of rats given AKW.

KEY WORDS: Rat milk electrolytes, Alkaline ionized water

INTRODUCTION

Because it is easily absorbed and excellent in regulating osmotic pressure, alkaline ionized water (AKW) is used for the purpose of supplementing losses of electrolytes and water during exercise and for preserving health. AKW is produced by electrolysis of an electrolyte solution through movement of cations to cathodes. Most of the cations which move to cathodes are hydrated with water molecules. Such water-hydrated cations are the physiologically active type rapidly absorbed and utilized by a living organism. With regard to experimental animals given AKW, Watanabe and Shirai (1990) reported that the body weight of the 3-week-old offspring of rats given AKW throughout gestation and lactation was significantly heavier than that of the control. Watanabe (1995) also reported that AKW had substantial biological effects on postnatal growth; for instance, the increase in intake of food and water in maternal rats given AKW. The body weights of offspring after day 14 of lactation in males, and after weaning in both males and females in the AKW group, were heavier than those in the tap water (TPW) group, and the postnatal morphological development was accelerated in the AKW group. However, there was no significant difference in the milk yield and the suckled milk volume between the two groups (Watanabe *et al.*, 1998).

The present study was designed to investigate whether the high concentrations of electrolytes in milk caused an increase in the body weight of the offspring.

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MATERIALS AND METHODS

Animal, housing and feeding

Eight-week-old female rats of the Sprague-Dawley strain, weighing 180-195 g (Jcl. SD., Clea Japan Inc., Tokyo) were maintained under conditions of 23±1℃ temperature, 40-60% humidity, and 14-hr illumination throughout the experiment. Five rats were housed in each plastic cage with wood-chip bedding, and reared on a diet (CE-2, Clea Japan Inc., Tokyo) and tap water (TPW) *ad libitum*. After 3 weeks of acclimation, animals without any abnormal findings were used. Copulation was induced by placing a proven-fertile male rat of the same strain in one cage made of aluminum containing 10 female rats with a regular estrous cycle confirmed by prior vaginal smears. A single male rat was used throughout the experiment. Vaginal smears were examined daily under the microscope to confirm the copulation. The day when sperm appeared in the vaginal smear was considered to be day 0 of gestation. Pregnant rats were distributed into two groups of 8 pregnant rats each and housed individually in the polycarbonate cages. The pregnant rats were given AKW, and the control rats were given TPW.

Electrolytic water ionizer

AKW was obtained by using an apparatus for producing the ionized water (Minekaru TBC-R 6103, Tokyo Seiden Co., Ltd., Tokyo). The apparatus brings the cation to the cathode and the anion to the anode based on the principle of electrolysis of an electrolyte solution. The amount of ions transferred varies with the amount of reacting substances, hydrogen ion concentration, and flow speed. The pH of AKW was 9.0, and the maximum flow-speed was 140 l/hr. The acidic water produced by the flow of anions to the anode was discarded. For the purification of AKW, TPW was electrolyzed without drugs.

Test for qualities of AKE and TPW

The pH, degree of alkalinity and electrolyte concentration of AKW and TPW were measured by the Japan Food Hygiene Association (Tokyo). The methods and items of the tests are summarized in Table 1.

Observation of delivery and nursing states

Animals of the test group were given AKW only from day 0 of gestation through day 14 of lactation, while those of the control group were given TPW *ad libitum*. The general and nursing states of the dams were observed daily. The pregnant rats were allowed to deliver spontaneously. The day when delivery was observed was designated as day 0 after delivery. Body weight was measured with an automatic balance for rats (Shin-maiko, Yamato Co. Tokyo) on days 0, 4, 7, 14 and 20 of gestation, and on days 0, 4, 7 and 14 of lactation. Intakes of food and water were measured on days 0, 4, 7, 10, 14 and 20 of gestation, and days 0, 4, 7, 10 and 14 of lactation.

Body weight of the offspring

On day 4 after birth, the litters were randomly adjusted to 8 pups comprised of 4 males and 4 females. The body weights of the offspring were measured with an electronic reading balance (Libro ED-200, Shimadzu Co. Tokyo) on postnatal days 4, 7, 10 and 14.

Milk samples

Eight offspring were separated from their dams from 8:00 a.m. to 4:00 p.m. on postnatal day 14, which is a day within the maximum lactation period (McGuire *et al*., 1995; Watanabe *et al*., 1998). The maternal rats were subcutaneously injected with 1 I.U. of oxytocin (Atonin-O, Teikoku Zoki Co. Ltd., Tokyo) at 4:00 p.m. Injection with oxytocin to the lactating rat and isolation of offspring for eight hr is the method applied when

Table 1. Qualities of alkaline ionized water (AKW) and tap water (TPW).

	AKW	TPW
pH [a, e]	8.7	7.3
Alkalinity (mg/l) [b]	50	38
Calcium (mg/l) [c]	20.1	17.5
Sodium (mg/l) [c]	8.6	7.8
Potassium (mg/l) [c]	2.1	1.7
Magnesium (mg/l) [c]	4.4	4.1
Zinc (mg/l) [c, e]	0.04	0.03
Iron (mg/l) [c, e]	<0.05	<0.05
Chloridion (mg/l) [d, e]	7.8	9.9

Water quality was analyzed by the Japan Food Hygiene Association, as recommended by the Minister of Health and Welfare based on the Japan Food Hygiene Act and the Japan Drug Act.

[a] pH meter method.

[b] Sulfuric acid neutralization titrimetry method.

[c] Atomic absorption spectrophotometry method.

[d] Silver nitrate titrimetry method.

[e] Method used for standardization of water quality based on the Japan Waterworks Act.

milk yield is measured by the body weight difference of offspring and dam before and after suckling (Grosvenor *et al.*, 1967; Mizuno and Satoh, 1970). At twenty min after injection with oxytocin, the dams were anesthetized with ether, then the udders were sterilized with 70% ethyl alcohol and massaged, and milk was collected. These milk samples were stored at 4℃ until analysis for content of electrolytes.

Milk electrolyte measurement

The milk electrolyte concentrations in the 8 mother rats given AKW and TPW on day 14 of lactation were analyzed by the Japan Food Research Laboratory Corporation (Tokyo) for contents of Ca, Na, K and Mg with the atomic absorption spectrophotometry method, and Cl with the microcoulometric-titrimetry method.

Hematological and serum biochemical examinations

Following the completion of all the tests, blood was collected from the jugular vein under ether anesthesia on day 14 of lactation. The numbers of erythrocytes and leukocytes were measured by the electronic counting method (Sysmex Microcell Counter CC-110, Toa Medical Electronics Inc., Kobe), hematocrit value was measured by the capillary method (Hematocrit H-2SF3, Toa Medical Electronics Inc., Kobe), hemoglobin value was measured by the cyanmethemoglobin method (Cannan, 1965), and blood glucose was measured by the method of *o*-toluidine (Hyvärinen and Nikkila, 1962). Serum was obtained by centrifugation at 1500 rpm for 5 min after the blood had been coagulated at 4℃ for 3 hr. Na and K concentrations of the serum were determined by a radiometer (KNA 1 sodium-potassium analyzer, Radiometer A/S Copenhagen, Denmark), and chloride by a chloride meter (CL-7 Chloride Counter, Hiranuma Sangyo Co., Ltd., Mito) according to the instrument manual instructions. Ca, Mg and inorganic phosphorus concentrations were determined by the methods of *o*-cresolphthalein complexone (Connerty and Briggs, 1966), xylidyl blue (Mann and Yoe, 1959) and molybdenum blue (Taussky and Shorr, 1953). Serum protein concentration was determined by a serum protein refractometer (Clinical Refractometer SPR-Ne, Atago Co., Ltd., Tokyo). Serum proteins were separated by cellulose acetate membrane electrophoresis (Separax-SP, Jookoo Co., Ltd., Tokyo), and the electrophoretograms were examined with a densitometer (Model PAN, Jokoo Co., Ltd., Tokyo).

Statistical analysis

All quantitative data were statistically analyzed by Student's *t* (*Welch*)-test. The 0.05 level of probability was used as the criterion for significance.

RESULTS

Qualities of AKW and TPW

The pH, alkalinity and Ca, Na, K, Mg, zinc, iron and chloridion concentrations of AKW and TPW are shown in Table 1. The concentrations of all electrolytes, excepting both chloridion and iron, were higher in AKW than in TPW.

Body weight and food and water consumption of maternal rats

Fig. 1 (A) shows the mean body weight of maternal rats given AKW or TPW during the gestation and lactation periods. No significant difference was noted between the AKW group and the TPW group. Fig. 1 (B and C) shows the mean maternal intakes of the food and water during the gestation and lactation periods. A significant increase in the water intake was noted on day 14 of gestation in the AKW group. Intakes of food and water on day 7 to day 14 of lactation were significantly increased in the AKW group.

Body weight of offspring

Fig. 2 shows the body weights of offspring. The mean body weight of males in the AKW group was significantly heavier than that in the TPW group. However, no significant difference in body weight of females was noted between two groups.

Hematological and serum biochemical determination

The hematological and serum findings are shown in Table 2. The numbers of erythrocytes and leukocytes, hematocrit value, hemoglobin and glucose concentrations in the AKW group are comparable to those in the TPW group. The values of the serum Ca, Na and K in the AKW group were significantly higher than those in the TPW group ($p<0.05$), and chloride in the AKW group was significantly lower than that in the TPW group ($p<0.05$). The Mg, inorganic phosphate, total protein and protein fractions were comparable among two groups.

Milk electrolyte

The results of the measurements of milk electrolyte concentrations are shown in Table 3. The concentrations of Ca, Na and K in milk were significantly higher in the AKW group than those in the TPW group. No significant differences were noted in Mg and Cl values between the AKW group and the TPW group.

DISCUSSION

We previously showed that an unusual increase in the body weight was observed in offspring born from the dams given AKW (Watanabe and Shirai, 1990; Watanabe, 1995; Watanabe *et al.*, 1997; Watanabe and Kishikawa, 1998). The present data confirmed the previous findings on AKW-induced acceleration of growth of offspring and suggested that the water-hydrated cations acted as a nutritional supplement.

AKW is produced by the movement of cations in the TPW towards the cathode through electrolysis. The amount of ions transferred depends on the quantity of the electrolytes, pH, and flow rate. Since only cations are transferred to AKW, the total amount of the cations in the AKW was increased (Table 1). Consequently, the alkalinity of AKW increased. A higher concentration of cations in AKW than TPW was shown by the measurement of electrolytes in the AKW and the TPW.

In the present study we determined differences in bioparameters, namely the milk electrolyte concentrations, between the AKW- and the TPW-treated groups in order to reveal the factor which causes the unusual increase in body weight of the offspring. The milk electrolyte concentrations in maternal rats on day 14 of lactation in the AKW group were significantly higher than those in the TPW group. No significant differences in Mg and Cl values in milk were observed between the two groups. The range of mean values for milk Na and K was from 85.1 to 110.4 mg/dl and from 136.5 to 210.6 mg/dl, respectively, in normotensive dam rats on postpartum days 8, 14 and 17 (McCarty and Tong, 1995). Gouldsborough and Ashton (1998) reported that milk Na and Ca of the normotensive Wister-Kyoto dam rats were 50.8±3.2 mg/dl and 184.6±8.6 mg/dl, respectively, on day 14 of lactation. Thus, milk concentration of electrolytes in rats varies considerably. The significance of these differences is not yet fully understood.

Since most of the cations transferred to AKW are hydrated with water molecules through electrolysis,

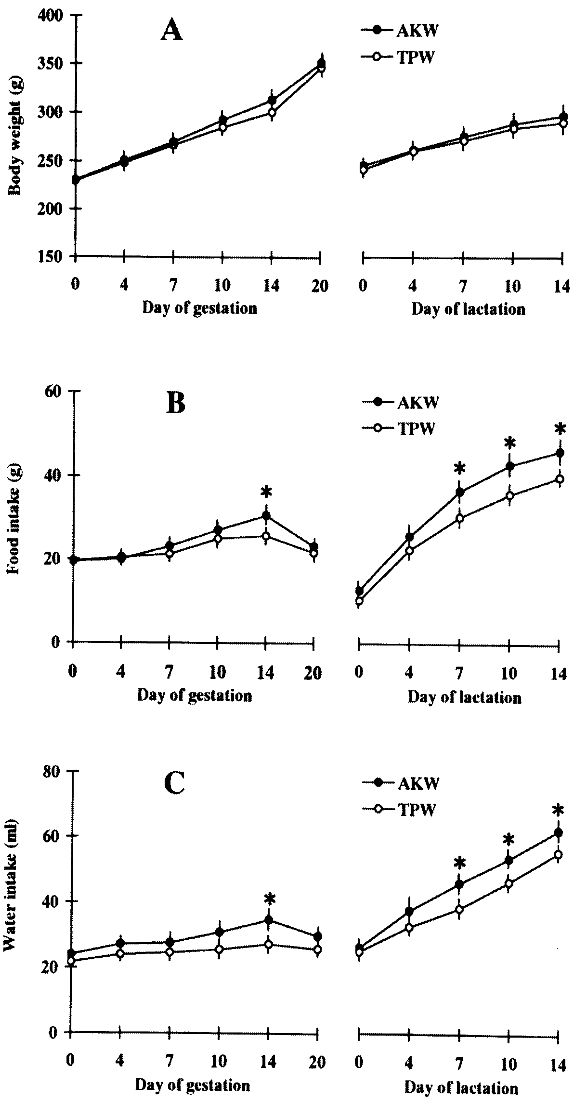

Fig. 1. Mean body weight, food and water intakes of maternal rats given AKW or TPW from day 0 of gestation to day 14 of lactation. A: Body weight, B: Food and C: Water intakes. Data are represented as mean±S.E. *Significantly different from the TPW group (p<0.05).

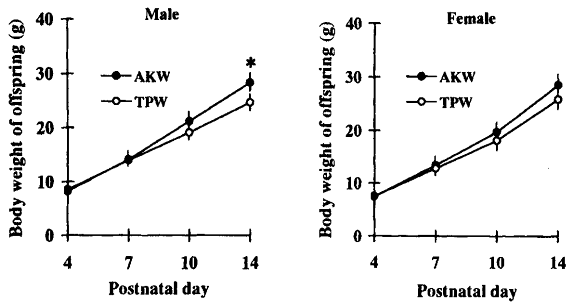

Fig. 2. Mean body weights of male and female offspring of rats given AKW or TPW from day 0 of gestation to day 14 of lactation (n=20). Data are represented as mean±S.E. *Significantly different from the TPW group (p<0.05).

Table 3. Concentrations of milk electrolytes in maternal rats given AKW or TPW from day 0 of gestation to day 14 of lactation.

	AKW	TPW
No. of rats	8	8
Calcium (mg/dl) a)	378.8 ± 58.8 *	332.7 ± 35.1
Sodium (mg/dl) a)	190.7 ± 33.0 *	162.7 ± 26.9
Potassium (mg/dl) a)	95.3 ± 22.6 *	77.0 ± 21.6
Magnesium (mg/dl) a)	23.2 ± 2.9	20.9 ± 2.4
Chloride (mg/dl) a)	186.4 ± 39.1	195.3 ± 33.0

a) Values are given as mean ± S.D.
* Significantly different from the TPW group ($p<0.05$).

they are rapidly absorbed and readily employed in an active form. The concentrations of Ca, Na and K of AKW were clearly higher than those of TPW, and the electrolyte concentrations in milk and serum of the dams given AKW were significantly higher than those of the dams given TPW (Table 2 and 3). It could be concluded that the rapid increase of the body weight of the offspring was caused by the fluctuation of the milk quality in response to the supply of AKW with a high concentration of cations during gestation and lactation.

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Table 2. Hematological and biochemical findings in maternal rats given AKW or TPW from day 0 of gestation to day 14 of lactation.

		AKW	TPW
No. of animals		8	8
Erythrocytes	($\times 10^4$mm^3) a)	766.7 ± 62.7	799.7 ± 69.0
Leukocytes	($\times 10^3$mm^3) a)	9.85 ± 1.83	9.20 ± 1.23
Hematocrit	(%) a)	42.0 ± 3.1	42.5 ± 3.5
Hemoglobin	(g/dl) a)	15.1 ± 1.6	15.7 ± 1.83
Glucose	(mg/dl) a)	87.5 ± 14.3	91.8 ± 19.1
Calcium	(mg/dl) a)	10.7 ± 0.6 *	10.1 ± 0.6
Sodium	(mEq/l) a)	142.8 ± 3.0 *	140.5 ± 2.9
Potassium	(mEq/l) a)	6.7 ± 0.4 *	5.7 ± 0.4
Chloride	(mEq/l) a)	102.5 ± 3.9 *	105.7 ± 3.7
Magnesium	(mg/dl) a)	3.4 ± 0.6	3.2 ± 0.4
Phosphorus	(mg/dl) a, b)	6.4 ± 0.5	6.4 ± 0.6
Total protein	(%) a)	7.4 ± 0.3	7.37 ± 0.43
Albumin	(%) a)	49.98 ± 2.33	50.02 ± 1.10
	(g/dl) a)	3.37 ± 0.20	3.69 ± 0.28
Globulin α-1	(%) a)	22.58 ± 1.15	21.32 ± 0.74
	(g/dl) a)	1.68 ± 0.14	1.56 ± 0.12
α-2	(%) a)	6.66 ± 1.12	6.48 ± 1.05
	(g/dl) a)	0.49 ± 0.08	0.47 ± 0.08
α-3	(%) a)	3.38 ± 0.70	3.72 ± 0.71
	(g/dl) a)	0.24 ± 0.06	0.26 ± 0.04
β	(%) a)	13.82 ± 1.38	13.91 ± 0.86
	(g/dl) a)	1.02 ± 0.10	1.02 ± 0.06
γ	(%) a)	3.51 ± 1.17	4.49 ± 1.25
	(g/dl) a)	0.25 ± 0.08	0.32 ± 0.08
A/G ratio a)		1.00 ± 0.09	1.00 ± 0.04

a) Values are given as mean ± S.D.
b) Inorganic phosphorus.
*Significantly different from the TPW group ($p<0.05$).

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