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# Aluminum concentrations in tissues of rats: effect of soft drink packaging

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**Aluminum is a commonly occurring trace element for which no nutritional requirements have been set. Some non-conclusive evidence exists suggesting a need of aluminum for growth, reproduction or health of man and animals. There is concern that exposure or consumption of aluminum may be toxic to humans and animals. The objective of the current study was to compare tissue levels of aluminum of rats fed soft drinks packaged in aluminum cans, glass bottles or distilled water. Thirty male weanling rats (Sprague-Dawley) were divided into three treatment groups of 10 rats each. All rats were fed rodent chow *ad libitum* throughout the study. Three different fluids, i.e. distilled water, diet soft drinks from aluminum cans and diet soft drinks from glass bottles, were fed for a period of 3 weeks. Aluminum contents of tissues were measured by atomic absorption spectrophotometry. Canned soft drink fed rats had significantly higher blood, liver and bone aluminum concentration than rats that were given glass bottled soft drink. There was a 69% higher bone aluminum concentration and 16% lower femur weight in rats fed aluminum canned soft drinks when compared with rats fed with distilled water.**

**Keywords:** aluminum, feces, rats, tissues

## Introduction

Aluminum is the most abundant metallic element within the lithosphere, occurring at about 8% by weight (Garrels *et al.* 1975, Hem 1986). Though aluminum is a strong hydrolyzing metal and is generally insoluble in neutral pH, under acidic or alkaline conditions the solubility of aluminum is enhanced (Stumn & Morgan 1970).

Past research has indicated that storage and/or cooking of acidic foods in aluminum containers results in the leaching of aluminum from the containers into foods, thereby increasing food aluminum content (Koning 1981, Greger 1985, Greger *et al.* 1985, Lione *et al.* 1985, Inoue *et al.* 1988, Pennington 1988). Other factors that contribute to the increase in aluminum content of foods include the type of aluminum utensil used (e.g. wrought or cast aluminum), pH of cooking water and the use of food additives (Miller *et al.* 1984, Greger *et al.* 1985). The objective of the current study was to compare tissue levels of aluminum of rats fed soft drinks packaged in aluminum cans, glass bottles or distilled water.

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## Materials and methods

Thirty male weanling rats (Sprague-Dawley strain) within a 2 g weight range were purchased from Harlan/Sprague-Dawley (Madison, WI). Upon arrival, the rats were housed in individual stainless steel cages equipped with one feed dish and fluid bottle per cage. Standard rat chow (Purina Rat Chow 5012; Ralston Purina, St Louis, MO) was fed for 7 days in order to accustom the animals to their new environment and to provide time for recovery from travel stress. Controlled temperature, humidity and lighting were maintained throughout the study.

After 7 days of adjustment, the rats were randomly assigned to three treatment groups (10 rats per treatment) so that initial mean weights of treatment groups were similar.

All rats were allowed rat chow (Ralston Purina Rodent Chow) *ad libitum* for 21 days; however, three different fluids were given to the three different treatment groups.

These were (i) distilled water, (ii) soft drinks from aluminum cans and (iii) soft drinks from glass bottles. The test beverages were mixed together in equal amounts and given *ad libitum* from standard bottle waterers. The specific brands of soft drinks used were determined on the basis of pH and preference. Aluminum concentrations of soft drinks packaged in aluminum cans, glass bottles, distilled water and ration were 0.47 $\mu g\,ml^{-1}$, 0.38 $\mu g\,ml^{-1}$, 0.023 $\mu g\,ml^{-1}$ and 0.11 $mg\,g^{-1}$ dry weight, respectively.

Diet drinks were employed so as to avoid impact on energy intake. Feed intakes were recorded on a weekly basis for each animal by determining the difference between feed weighed at the beginning and end of each week. Spillage was collected, weighed and subtracted from initial feed weight. Fluid intake was recorded and fresh fluid was given daily. Body weight was recorded throughout the study on a weekly basis using an Ohaus Autogram 1000 balance (Ohaus Scale, Union, NJ). Feces were collected daily and composited into 7 day lots. Fecal samples were separated from wasted feed, weighed to determine weekly output, dried, and reweighed and ground to a fine powder. Samples were then ashed in preparation for aluminum determination.

At the end of the study, the animals were fasted for 12 h, weighed in random order and anesthetized with carbon dioxide from dry ice. Blood was collected by heart puncture. The right femur was surgically removed from each animal. The liver and brain of each animal was excised and frozen using dry ice. Liver, brain, fecal, test beverages and feed aluminum were measured using a Varian Techtron Atomic Absorption Spectrophotometer Model 1275. Whole blood aluminum was determined by using a graphite furnace atomizer (Varian Techtron GTA-95). Fecal, test beverages and feed samples were ashed at 600 °C for 8 h, digested with nitric acid and diluted with distilled deionized water prior to analysis. Whole blood samples were diluted 1:1 with 0.1% tritonx solution and then analyzed directly. Liver and brain samples were digested with nitric acid and diluted with deionized distilled water prior to analysis. A primary standard for aluminum (Fisher Scientific, 1000 p.p.m.) was used to calibrate the instrument and a standard curve was obtained for various dilutions. All aluminum concentrations were performed in triplicate. Response criteria measurements included bone, brain, liver, fecal and whole blood aluminum levels.

Statistical analysis of data was completed by use of Statistical Analysis System (SAS) with the advice of the Biometrics Laboratory, Institute of Agriculture and National Resources, University of Nebraska-Lincoln.

The analysis of variance procedure and pre-planned comparisons were performed in order to detect possible variations resulting from beverage treatment. To determine possible difference between mean responses, the Least Square Means test was performed. Regression analysis was conducted to determine if aluminum intake had an effect on weight gain.

## Results and discussion

As shown in Table 1, rats fed the same diet but different fluids showed no significant differences in weight gain ($P = 0.3967$) and feed intakes by rats of the different treatment groups were not significantly different. Regression analysis showed that beverage type did not affect weight gain.

Rats fed soft drinks packaged in bottles had a significantly greater consumption of the beverage when compared with distilled water fed rats ($P = 0.0022$). A similar situation was observed when rats were fed soft drinks packaged in aluminum cans ($P = 0.0006$). There was no statistically significant difference in the consumption of beverages packaged in either cans or glass bottles.

This could be attributed to the sweetener present in the soft drinks, which enhanced a greater consumption of the beverage, unlike distilled water which was devoid of any sweetener. Since the test beverages were diet drinks (i.e. soft drinks in cans and bottles), they did not contribute significantly to the weight gain of the rats.

The total feed intakes of rats were not affected by beverage treatment and this, in turn, had no effect on the weight gain of the animals. Feed intake by rats is usually directly proportional to fluid intake but this relationship did not hold true in the present study.

As shown in Table 2, mean whole blood aluminum values for rats fed distilled water, soft drinks

**Table 1.** Mean weight gain, fluid consumption and total feed intake of weanling rats fed glass bottled diet soft drinks, aluminum canned diet soft drinks or distilled water

| Parameter | Source of fluid | | |
|---|---|---|---|
| | bottle | aluminum can | distilled water |
| Weight gain (g) | 141.6 ± 12.8 [a] | 142.0 ± 9.29[a] | 137.1 ± 20.48[a] |
| Beverage consumption (ml) | 739.2 ± 55.57[a] | 751.4 ± 59.07[a] | 655.9 ± 50.47[b] |
| Total feed intake ($g\,rat^{-1}\,21\,days^{-1}$) | 369.6 ± 16.56[a] | 370.7 ± 16.51[a] | 363.8 ± 20.02[a] |

Values with different letter superscripts are significantly different from one another ($P < 0.05$).

**Table 2.** Mean blood, brain, liver and bone aluminum concentrations of weanling rats fed glass bottled diet soft drinks, aluminum canned diet soft drinks or distilled water

| Parameter | Source of fluid | | |
|---|---|---|---|
| | bottle | aluminum can | distilled water |
| Whole blood ($\mu g\,dl^{-1}$) | $51.22 \pm 15.41^{a}$ | $64.07 \pm 38.23^{a}$ | $39.84 \pm 26.50^{a}$ |
| Brain ($\mu g\,g^{-1}$) | $0.26 \pm 0.19^{ab}$ | $0.42 \pm 0.15^{a}$ | $0.19 \pm 0.10^{b}$ |
| Bone ($\mu g\,g^{-1}$) | $1.93 \pm 0.97^{b}$ | $4.39 \pm 1.36^{a}$ | $2.59 \pm 1.11^{b}$ |
| Liver ($\mu g\,g^{-1}$) | $1.11 \pm 0.44^{b}$ | $2.04 \pm 1.21^{a}$ | $2.03 \pm 0.73^{a}$ |

Values with different letter superscripts are significantly different from one another ($P < 0.05$).

packaged in aluminum cans and soft drinks packaged in glass bottles were 39.84, 64.07 and 51.22 $\mu g$ $day^{-1}$, respectively. Although mean whole blood values were not statistically different, numerical trends are obvious. Differences between whole blood aluminum concentrations of rats fed aluminum canned soft drinks and those fed distilled water approached significance ($P = 0.0661$) but those between animals fed bottled soft drinks and distilled water did not ($P = 0.3764$). Numerical differences were also observed in rats fed either canned or bottled soft drinks. These findings support the work of other researchers (Kaehny *et al.* 1977, Bernuzzi *et al.* 1989).

Brain aluminum levels, as shown in Table 2, were significantly higher in rats fed canned soft drinks when compared with those of distilled water fed rats ($P = 0.0235$). However, animals fed the bottle soft drinks were not demonstrated to have higher brain aluminum concentrations than those given distilled water ($P = 0.4770$). Numerically, values of the bottled fed animals tended to be lower ($P = 0.1045$) than those fed aluminum can packaged soft drinks. This could be attributed to the fact that the acidic beverages enhanced the leaching of aluminum from cans and thereby increased the concentration of aluminum in the brain (Chan *et al.* 1983).

Mean bone aluminum values, for rats fed the same dietary manipulations, were 2.59 (distilled water), 4.39 (aluminum can) and 1.93 (bottle) $\mu g\,g^{-1}$ dry weight, respectively. Bone aluminum levels were found to be significantly higher in can fed rats when compared with bottled or distilled water fed rats. Generally more aluminum has been found to accumulate in bone than in soft tissues (Ondreicka *et al.* 1966, Greger *et al.* 1985, 1986, Slanina *et al.* 1985). These results indicate that bone is extremely sensitive to variations in aluminum intake; hence, it is a highly desirable test tissue to use in evaluations of dietary aluminum. The concentraton of bone aluminum in bottle fed rats was significantly lower to that of can fed rats ($P = 0.0001$).

Mean liver aluminum concentrations (Table 2) were numerically lower in rats fed bottled soft drinks ($1.11\ \mu g\,g^{-1}$) than for those fed other treatments. Canned soft drink fed rats showed the highest mean liver aluminum concentration ($2.04\ \mu g\,g^{-1}$). There was a significantly higher concentration of aluminum in the livers of rats fed canned soft drinks than those fed bottled soft drinks ($P = 0.0216$).

The type of beverage had a significant effect on the femur bone weights of rats ($P = 0.0424$). Mean right femur bone weight of rats fed bottled soft drinks, canned soft drinks or distilled water were 0.306, 0.269 and 0.321 g (Table 3). From pre-planned comparisons, it was shown that there was a statistically significant difference in the bone weight of rats fed canned soft drinks and distilled water fed rats ($P = 0.0156$), and a tendency between rats fed canned soft drinks and bottled soft drinks ($P = 0.0718$). A decrease in bone weight is suggestive of osteoporosis, a serious problem frequently found in elderly women.

Mean fecal aluminum excretions of rats for weeks 1, 2 and 3 are listed on Table 4. During time periods I, II and III the different dietary treatments did not have a significant effect of fecal aluminum excretions. Pre-planned comparisons indicated that

**Table 3.** Mean bone weight (dry fat free) of weanling rats fed glass bottled diet soft drinks, aluminum canned diet soft drinks or distilled water

| Parameter | Bottle | Aluminum can | Distilled water |
|---|---|---|---|
| Bone weight (g) | $0.31 \pm 0.03^{ab}$ | $0.27 \pm 0.05^{b}$ | $0.32 \pm 0.05^{a}$ |

Values with different letter superscripts are significantly different from one another ($P < 0.05$).

**Table 4.** Effect of beverage treatment on mean fecal aluminum concentration ($\mu g\, g^{-1}$) in weanling rats

| Period | Bottle | Aluminum can | Distilled water |
|---|---|---|---|
| Week I | 7.7 ± 1.99 | 8.5 ± 2.10 | 7.4 ± 1.83 |
| Week II | 10.1 ± 1.88 | 11.7 ± 3.60 | 10.6 ± 2.42 |
| Week III | 13.3 ± 3.30 | 17.1 ± 5.98 | 14.5 ± 3.92 |

between periods I, II and III there was a statistically significant difference in fecal aluminum excretion ($P = 0.0001$) though within period this was not exhibited. Larger rats consumed more feed and fluid than did smaller rats, hence, higher excretions of aluminum would be expected in the last week than the first week of the project.

Aluminum absorption within the gastrointestinal tract is dependent on the chemical form of the ingested aluminum. In addition, the ingestion of aluminum compounds with either fruit juices or citric acid caused a marked increase in the gastrointestinal absorption of healthy subjects (Slanina *et al*. 1986, Weberg & Berstad 1986). Since the bottled soft drinks were as acidic as were those packaged in cans, this may have contributed to aluminum absorption from the diet. Glass also contains aluminum as a contaminant although the concentration is much smaller than that of aluminum cans. However, aluminum cans used for soft drinks are lined while glass bottles are not. This may explain the relatively high concentrations of aluminum in both types of soft drinks.

In conclusion, bone tissue, apparently, was most adversely affected by consumption of aluminum from soft drink containers. The 69% increase in bone aluminum concentration and 16% decrease in femur weight when rats were fed the aluminum canned soft drinks rather than distilled water may be of concern.

## References


Bernuzzi V, Desor D, Lehr, PR. 1989 Developmental alterations in offspring of female rats orally intoxicated by aluminum chloride or lactate during gestation. *Teratology* **40**, 21.

Chan YL, Alfrey AC, Posen L, *et al*. 1983 The effect of aluminum on normal and uremic rats: tissue distribution, vitamin D metabolites and quantitative bone histology. *Calcified Tissue Int*. **35**, 344.

Garrels RM, Mackenzie FT, Hunt C. 1975 *Chemical Cycles and the Global Environment*. W Kaufmann, Los Altos, CA, USA.

Greger JL. 1985 Aluminum content of the American diet. *Food Technol* **39**, 73.

Greger JL, Bula EN, Gum ET. 1985 Mineral metabolism of rats fed moderate levels of various aluminum compounds for short periods of time. *J Nutr* **115**, 1708.

Greger JL, Bula EN, Gum ET. 1986 Mineral metabolism of rats fed various levels of aluminum hydroxide. *Biol Trace Elem Res* **9**, 67.

Greger JL, Goetz W, Sullivan D. 1985 Aluminum levels in foods cooked and stored in aluminum pans, trays and foil. *J Food Protect* **48**, 772.

Hem JD. 1986 Geochemistry and aqueous chemistry of aluminum. *Kidney Int* **29**, 53.

Inoue T, Ishiwata H, Yoshihira K. 1988 Aluminum levels in food-stimulating solvents and various foods cooked in aluminum pans. *J Agric Food Chem* **36**, 599.

Kaehny WD, Hegg AP, Alfrey AC. 1977 Gastrointestinal absorption of aluminum from aluminum containing antacids. *New Engl J Med* **296**, 1389.

Koning JH. 1981 Aluminum pots as a source of dietary aluminum (letter). *New Engl Med* **304**, 172.

Lione A, Allen PV, Smith JC. 1984 Aluminum coffee percolators as a source of dietary aluminum. *Food Chem Toxicol* **22**, 265.

Miller RG, Kopler FC, Felty KC, *et al*. 1984 The occurences of aluminum in drinking water. *J Am Water Works Ass* **76**, 84.

Ondreicka R, Ginter E, Kortus J. 1966 Chronic toxicity of aluminum in rats and mice and its effect on phosphorus metabolism. *Br J Ind Med* **23**, 305.

Pennington JAT. 1988 Aluminum content of foods and diets. *Food Additives Contaminants* **5**, 166.

Slanina P, Frech W, Bernhardson A, *et al*. 1985 Influence of dietary factors on aluminum absorption and retention in the brain and bone of rats. *Acta Pharmacol Toxicol* **56**, 331.

Slanina P, Frech W, Ekstrom G, *et al*. 1986 Dietary citric acid enhances absorption of aluminum in actacids. *Clin Chem* **32**, 539.

Stumn W, Morgan JJ. 1970 *Aquatic Chemistry*. Wiley-Interscience, New York.

Weberg R, Berstad A. 1986 Gastrointestinal absorption of aluminum from single doses of aluminum containing antacids in man. *Eur J Clin Invest* **16**, 428.