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## **Effects of alkaline ionized water on formation & maintenance of osseous tissues**

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Effects of calcium alkaline ionized water on formation and maintenance of osseous tissues in rats were examined. In the absence of calcium in the diet, no apparent calcification was observed with only osteoid formation being prominent. Striking differences were found among groups that were given diets with 30% and 60% calcium. Rats raised by calcium ionized water showed the least osteogenetic disturbance. Tibiae and humeri are more susceptible to calcium deficiency than femora. These results may indicate that calcium in drinking water effectively supplements osteogenesis in case of dietary calcium deficiency. The mechanism involved in osteoid formation such as absorption rate of calcium from the intestine and effects of calcium alkaline ionized drinking water on maintaining bone structure in the process of aging or under the condition of calcium deficiency is investigated.

Osteoporosis that has lately drawn public attention is defined as "conditions of bone brittleness caused by reduction in the amount of bone frames and deterioration of osseous microstructure." Abnormal calcium metabolism has been considered to be one of the factors to contribute to this problem, which in turn is caused by insufficient calcium take in, reduction in enteral absorption rate of calcium and increase in the amount of calcium in urinal discharge. Under normal conditions, bones absorb old bones by regular metabolism through osteoid formation to maintain their strength and function as supporting structure. It is getting clear that remodeling of bones at the tissue level goes through the process of activation, resorption, reversal, matrix synthesis and mineralization. Another important function of bones is storing minerals especially by coordinating with intestines and kidneys to control calcium concentration in the blood. When something happens to this osteo metabolism, it results in abnormal morphological changes. Our analyses have been focusing mostly on the changes in the amount of bones to examine effects of calcium alkaline ionized water on the reaction system of osteo metabolism and its efficiency. Ibis time, however, we studied it further from the standpoint of histology. In other words, we conducted comparative studies on morphological and kinetic changes of osteogenesis by testing alkaline ionized water, tap water and solution of lactate on rats.

Three week old male Wistar rats were divided into 12 groups by conditions of feed and drinking water. Feeds were prepared with 0%, 30%, 60% and 100% of normal amount of calcium and were given freely. Three types of drinking water, tap water (city water, about 6ppm of Ca), calcium lactate solution (Ca=40ppm) and alkaline ionized water (Ca =40ppm, pH=9, produced by an electrolyzer NDX 4 LMC by Omco OMC Co., Ltd.) were also given keely. Rats' weight, amount of drinking water and feed as well as the content of Ca in drinking water were assayed every day. On the 19th and 25th days of testing, tetracycline hydrochloride was added to the feed for 48 hours so as to bring its concentration to 30mg/kg. On the 30th day, blood samples were taken under Nembutal anesthesia, and tibiae, humeri and femora were taken out to make non decalcified samples. Their conditions of osteoid formation and rotation were observed using Villanueva bone stain and Villanueva goldner stain.

Three groups that were given different types of drinking water and the same amount of Ca in the feed were compared to find out no significant difference in the rate of weight gain and intakes of feed and drinking water. Alkaline ionized water group had significantly greater amount of tibiae and humeri with higher concentration of calcium in the bones.

The group of 0% calcium in the feed saw drastic increase in the amount of osteoid. There was not much difference by types of drinking water. Almost no tetracycline was taken into tibiae and humeri, although a small amount was identified in femora. As a result, osteogenesis went as far as osteoid formation, but it was likely that decalcification has not happened yet, or most of newly formed bones were absorbed.

As to the groups of 30% and 60% calcium in the feed, increase in the area of tetracycline take in was more identifiable with higher clarity in descending order of alkaline ionized water, calcium lactate solution and tap water groups. Especially in case of tap water group, irregularity among the areas of tetracycline take in was distinctive. The group of 100% calcium in the feed saw some improvements in osteogenesis in descending order of alkaline ionized water, calcium lactate solution and tap water. In any case, bone formation seemed to be in good condition at near normal level.

Alkaline ionized water was regarded to be effective for improvements of osteogenesis under the conditions of insufficient calcium in the feed. Also, the extent of dysosteogenesis differed by the region. That is, tibiae and humeri tend to have more significant dysosteogenesis than femora.

In addition, there is a possibility that osteo metabolism varies depending on enteral absorption rate of calcium, adjustment of discharge from kidneys and functional adjustment of accessory thyroid in the presence of alkaline ionized water. We are now studying its impact on calcium concentration in the blood. We are also examining whether it is possible to deter bone deterioration by testing on fast aging mouse models.