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Assessment of Osteogenesis Enhancement in Rats Using Bone Densitometry: An *In Vivo* Study

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Abstract

BACKGROUND: Human survival depends on the availability of water. Safe drinking water is a fundamental human right as well as a basic need for a good health. At the mineral and organic levels of water composition, exposure to electromagnetic fields can cause considerable changes in the quality of drinking water. As a result, water is more easily absorbed into the cell walls throughout the body, making it better suited for organ growth and development. When drunk on a daily basis, magnetic water causes dramatic changes in bone mineral density and content. Due to the necessity for distinct beam filtering and near-perfect spatial discography of the two attenuations, DualEnergy X-ray absorptiometry (DXA) was included with general-purpose X-ray systems. In rats that drink magnetized water on a daily basis, the DXA can be used to calculate the weight and area of all head compartments.

AIM: By employing a bone densitometry scan on the head regions of rats, this study aims to assess the changes in the rat's skull bone measures (area, weight, bone mineral content, and bone mineral density) after feeding the animal with magnetized water.

METHODS: This study involved 30 adult male rats (6 weeks old). For 30 days, 15 rats were given a magnetized water to drink and used as experimental animals. For comparison purposes, the remaining 15 rats were given regular water for 30 days. After the experimental procedure was done, each rat's head was scanned with a DXA to determine bone weight, area, bone mineral content, and bone mineral density. Before drinking water, each rat in both the experimental and control groups was examined. The head of each rate was scanned using a DXA to get the values of bone: Weight, area, mineral density, and mineral content after 30 days of consuming magnetized water. The four bone measures were statistically compared in the experimental and control rats, p-values below 0.05 were considered significant.

RESULTS: Comparing to control rats, experimental rats showed a significant increase in the bone's weight and area of the skull ($p < 0.05$, Wilcoxon test). Bone marrow content and bone marrow density enhanced significantly as well in experimental rats after drinking the magnetized water for 30 days.

CONCLUSIONS: Drinking water that has been subjected to electromagnetic fields for 30 days can improve bone marrow content and density in rats. Drinking magnetized water also has a substantial impact on bone mass and area.

Introduction

The structure and function of bone are highly specialized and complicated [1]. The soft compartment of the skull, which is made up of highly active, mineralized, and vascular connective Tissue, is protected and supported by the hard compartment of the skull, which is widely recognized for its pliability, hardness, and capability to offer protection to the underlying structures [2]. Body composition is critical and important for a full assessment of nutritional health. Body mass and weight indices are well-known health indicators [3], [4]. Estimating the change in body weight "predominantly weight loss" has been a dominant finish point for the assessment of nutritional status during the course of a clinical condition. In fact, nutritional status is beneficial when significant changes are observed [4]. Bone's unique mechanical qualities come from its composite nature. Bone is made up of two basic components: An organic matrix (mainly Type I collagen) and a mineral matrix (hydroxyapatite crystals embedded in collagen fibers). It has been established that the mineral component contributes significantly to bone strength, but the organic matrix is principally responsible for bone toughness and plastic deformation [5].

Physiological consequences of the electromagnetic field interactions with living beings have sparked a rise in attention in recent years. Industrial and electromagnetic waves continue to have an impact on all living organisms. Domestic sources of cell activity induced by extremely low frequency magnetic field "ELF-MF" partially illustrate how biological systems interact with electromagnetic fields [6], [7]. After exposure to a magnetic field, considerable changes in pH of the dissolved solids, total hardness, salinity, conductivity, dissolved oxygen, vaporized temperature, minerals, organic matter, and total bacteria count are detected in magnetized water for people and animals [8]. It will then aid in the acceleration of normal penetration by promoting cell wall penetration. The creation of numerous organs depends on the diffusion of magnetized water. "Magnetic water" is defined as "water evaporating from magnetic tubes and a magnet being inserted into it, causing the water's properties to become very active and resulting in a high oxygen ratio, dissolved salts velocity, and amino acid velocity in water" [2], [6]. Fresh water is referred to as "dead water," but following sterilization and magnetization, it becomes a "living water" [2], [6], [9]. Properties of the treated water could be modified to make it more energized and active with a high pH toward mild alkaline and germ-free [10].

Figure 1: Bone densitometry

The best fracture risk predictor is DXA (Dual-Energy X-ray Absorptiometry), the most basic approach for assessing body weight and area composition [11], [12]. For assessing molecular body composition, DXA is a commonly used method [13], [14], (Figures 1 and 2). As illustrated in Figure 2, bone mass, area, and density, as well as fat and fat-free soft tissues, can all be assessed at levels that affect the entire body and the entire district. DXA has the advantage of primarily measuring bone and muscle (except for a slight amount of connective tissue and skin) [15]. It is also a very precise method for determining body structure. In fact,

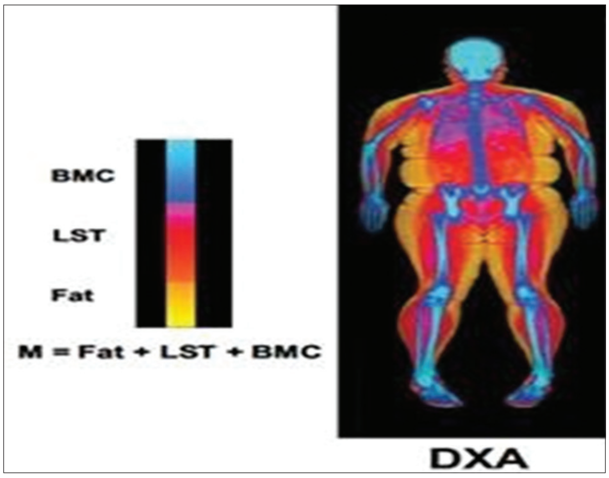

Figure 2: Selected body composition, components measured by DXA [13], [14]

its overall accuracy surpasses any other body structure technology [16]. Low-energy X-rays with two unique photon energy levels flow through the body during a DXA scan and are caught by a photon detector, which calculates how much energy is taken (attenuation) by soft and hard tissue at each pixel [2], [6]. The DXA results are influenced by body thickness [17]. Greater tissue thickness of more than 25 cm causes increased photons of energy attenuation, resulting in a disproportionate shift to high-energy photons, which can lead to a fat mass in stocky people [18]. To produce very low-radiation X-rays, the two DXA low-energy X-ray beams are used. An X-ray source generates a large and brief DXA to distinguish between soft tissue and bone. It can distinguish between bone tissues and enables for local body structure measurements. It is safe for frequent interventions and is quick and painless with high precision and accuracy [2]. It is important to notice that DXA's inability to distinguish between visceral and subcutaneous fat, intramuscular fat, and LST (which includes muscle and organs) can be a functional restriction in clinical situations. In conclusion, orthopedic implants may create fragments that impact DXA capabilities, resulting in inexact soft-tissue differentiation. The overall impact of the same objects on measurements of whole-body conformation is recognized to a minor degree [16], [19].

It is well documented that a decreased body weight is linked to low bone mass and eventually to osteoporosis. Bone area (BA) refers to the projected area of the bone into the imaging plane, which is generally quantified in cm^2. Bone marrow content (BMC) is the hydroxyapatite "$Ca_{10}(PO_4)_6(OH)_2$" component of bone mineral mass, usually expressed in grams. It is worth mentioning that BMC lacks the majority of bone's organic components (marrow, collagen, etc.). As a result, only thatched bone models can be used to assess the accuracy of bone containment in organic substance as indicated by Ca^{+2} and pho_4 ions. Bone mineral density (BMD) is defined as the mineral mass of bone per unit area (g/cm^2). This is where the difference between true volume density and actual density is defined. DXA cannot identify "volume density," or the mineral mass per unit bone volume. A BMD is calculated using the formula aBMD = BMC/BA (g/cm^2) [20]. When osteoporosis occurs, all those four measures are adversely impacted. We employed them in this study to assess the effect of consuming magnetic water and to look into the possibility of employing the magnetized water to treat osteoporosis.

Materials and Methods

All experimental procedures were carried out with the consent of ethical guidelines defined by the scientific committee of the institute of medicine faculty/university of Diyala/Iraq. Data from thirty adult male rats (6–8) weeks of age were collected and compiled at university of Diyala from November 1, 2017 to May 1, 2018. The experimental procedures were carried out on 15 rats, with the remaining 15 rats serving as controls. Each rat was kept in its own plexiglass cage with free access to food and water. Temperature and humidity at the experimental room were set to be 2024°C and 50%, respectively. Water is poured into the magnetic device's open side, which is closely connected to the tap water container on the other side. The voltage regulator and timer are then modified to determine the magnetization cycle timing, after which the electric continuous power point is turned on. After a 60-s exposure, the rats fed the water collected by the magnetic device. After that, values of head parts (weight, area, bone marrow density, and bone marrow content) were collected using the DXA scans.

Statistical analysis

All statistical calculations and analyses were performed using MATLAB (R2015a). All data were expressed as the mean ± SEM. The effects of magnetized water on the weight, area, BMD, and BMC were statistically evaluated using the Wilcoxon rank-sum test; a false positive error probability p under 0.05 was considered significant.

Results

Weight and area before and after drinking the regular and magnetized water over 30 days for control and experimental rats are compared in Figures 3 and 4 as daily averages pooled for all 15 rats. Regarding the weight parameter, as expected control and experimental rats weight increased significantly (Wilcoxon test, p = 0.02 and 0.013) consecutively after the 30 days of the experimental procedure, Figure 3. Comparing experimental rats to the controls, our data showed that the experimental rats significantly gained weight over the 30 days (Wilcoxon test, $p < 0.05$). Bone area in control and experimental groups showed an increase in the area after drinking the regular and magnetized water for 30 days but was significant only for the experimental group (Wilcoxon test, $p < 0.05$), Figure 4. Compared to controls, the estimated area of the experimental rats increased significantly after drinking the magnetized water for up to 4 weeks, Figure 4.

After that, we looked at the changes in bone marrow content (BMC) and bone marrow density (BMD) in both groups before and after applying the water drinking procedure. While the range of bone marrow content (BMC) was 22–30 g for control rats, it was 19–23 g for the experimental rats. Our results showed that the BMC increased in the control and experimental rats after they drank the regular and magnetized water for 30 days but the increment was significant (Wilcoxon test, $p < 0.05$) only in the experimental rats, Figure 5. Comparing the BMC of the experimental rats after the 30 days to that of controls, our data showed that there was a significant increase in its value (Wilcoxon test, $p < 0.05$) as shown in Figure 5.

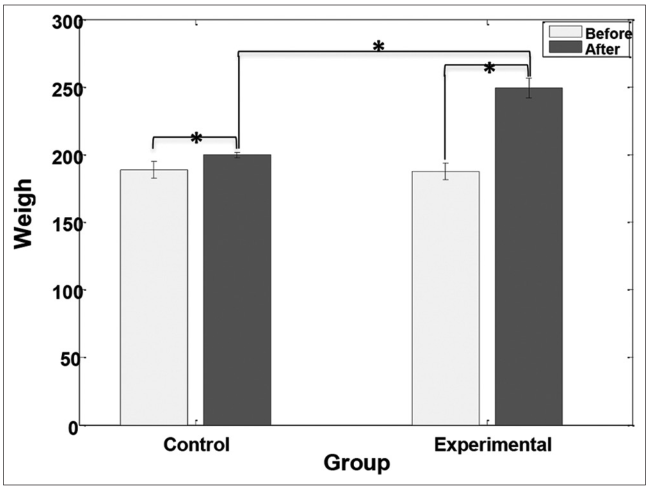

*Figure 3: Weight changes in control and experimental rats after drinking regular and magnetized water for 30 days. The weight of controls and experimental rats increased significantly (Wilcoxon test, $p < 0.05$). Comparing to controls, experimental rats weight increase d significantly after the 30 days. *Indicates a significant difference. Weight measured in gram*

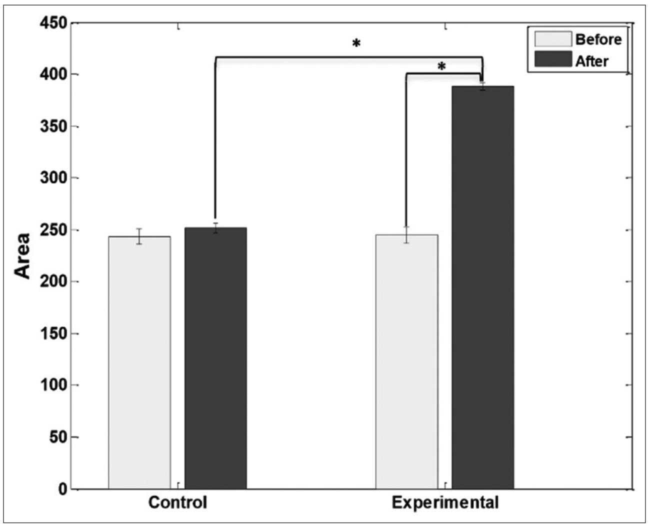

*Figure 4: Area changes in control and experimental rats after drinking regular and magnetized water for 30 days. The Group area of controls and experimental rats increased but was significant only in experimental rats (Wilcoxon test, $p < 0.05$). Comparing to controls, area of the experimental rats increased significantly after the 30 days. *indicates a significant difference. Area measured in cm^2*

Bone marrow density (BMD) is an important factor in evaluating the effect of the magnetized water.

Looking at the data of each rat, BMD increased after drinking the regular and magnetized water for up to 4 weeks. As an average data for all rats, our data showed that there was a significant increase in the BMD in both groups after the 30 days of drinking regular and magnetized water (Wilcoxon test, $p < 0.05$).

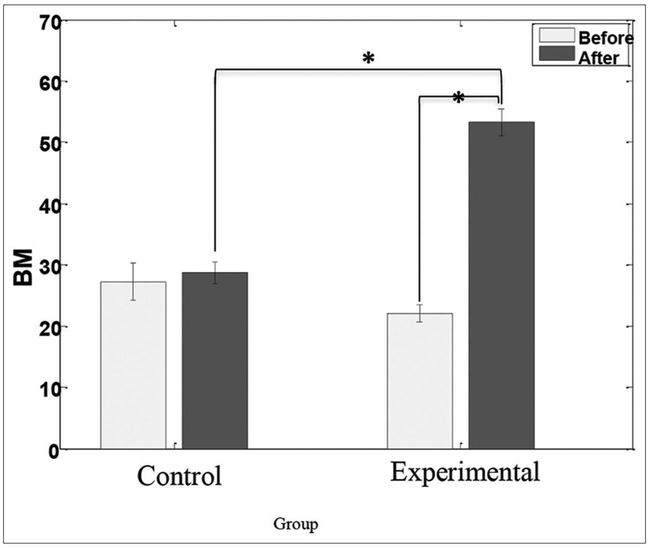

*Figure 5: Changes in bone marrow content (BMC) in control and experimental rats after drinking regular and magnetized water for 30 days. The BMC of controls and experimental rats increased but was significant only in experimental rats (Wilcoxon test, p < 0.05). Comparing to controls, BMC of the experimental rats increased significantly after the 30 days. *indicates a significant difference. BMC measured in gram*

Bone marrow density was significantly greater (Wilcoxon test, $p < 0.05$) for the experimental rats than the controls after 30 days of drinking magnetized water as shown in Figure 6.

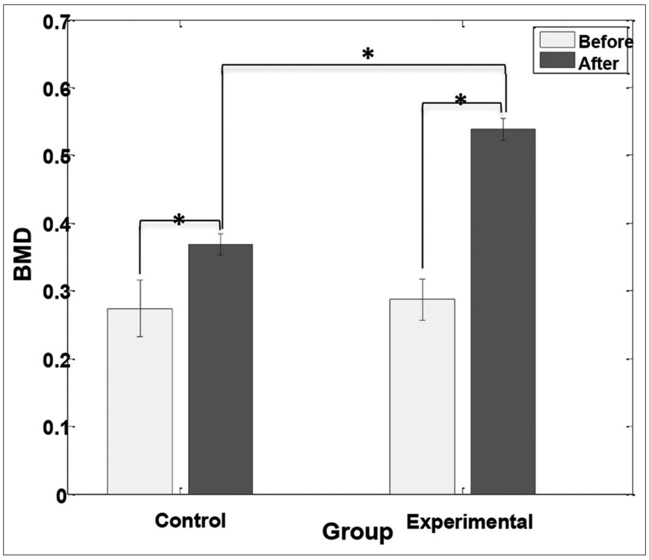

*Figure 6: Changes in bone marrow density (BMD) in control and experimental rats after drinking regular and magnetized water for 30 days. The BMD of controls and experimental rats increased significantly in control and experimental rats (Wilcoxon test, p < 0.05). Comparing controls, BMD of experimental rats increased significantly after the 30 days. *indicates a significant difference. BMD measured in g/cm^2*

Discussion

The aim of this study was to find out how drinking magnetized water could affect bone marrow content and density in rats as a way to aid growth, increase bone weight and mass, and use it to facilitate bone healing and provide a simple treatment for osteoporosis. Exposing water to strong magnetic fields affected its mineral contents and its effects depending on the strength of the magnetic field and exposure time. When exposed to a magnetic field, water quality improved, with substantial changes in PH which can reach 9.2, total dissolved solids, conductivity, salinity, minerals, and organic matter agree with [2], [10]. In the present study, 15 adult male rats were treated with magnetic water for 30 days. The results showed a significant increase in the following bone measurements: Area, weight, bone marrow content, and bone marrow density after consuming the magnetized water over 30 days. This explains the improvement in the four bone measurements that we observed in our findings. The results agree with those done by the previous studies [20].

The whole repairing construction of a broken bone takes roughly 4 weeks, passing through the phases of osteoid development, calcification, trabecular bone production, and bone hardening. Because of this, we decided to run our experiment for 30 days. The experimental group's lean soft-tissue mass (LSTM) and fat soft-tissue mass (FSTM) of the skull exhibited a highly significant increase in area and weight compared to the control group after consuming magnetized water. Thus, the exposure to a magnetic field for 30 days is considered as an efficient strategy to promote osteogenesis in a short period of time.

The beneficial role of magnetize water is influenced by hormonal changes in female laboratory animals that cause osteoporosis. As a result, this study was limited to male rats to emphasize the importance of the process of osteogenesis. Although DXA is an efficient approach for assessing bone density, it is limited in its ability to diagnose certain locations and anomalies, such as the spine after previous surgery. The existence of compression fractures in the spine or osteoporosis can make the test less accurate; in these circumstances, a CT scan can be used. We are currently evaluating the concentration of magnetic water content, which may be useful to this research.

Conclusion

Drinking magnetized water for 30 days has a substantial impact on the bone measures (area, weight, bone mineral content, and bone mineral density) in rats. The impact could be beneficial in the treatment of osteoporosis patients.

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