Bone Changes Due to Hyperbaric Exposure

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Abstract. Based on the hypothesis that bone calcification is promoted by loading physical pressure, changes in the microstructure of the bone under hyperbaric conditions were analyzed by imaging technology. Hyperbaric exposure was carried out for two weeks at 2 atm (equal to the pressure at a depth of water of 10 m) which was achieved using a mixed gas of helium and oxygen (He:O₂ 88%:12%) in which the oxygen partial pressure was maintained at constant (PO₂: 0.21 bar). In image technological analysis, the growth and development of the bone were evaluated at different stages using Digital Magnification Radiography (DMR) images and based on changes in the X-ray absorption ratio. DMR images after hyperbaric exposure showed calcification in the heads of long bones (humeri, femora, and tibiae) in mice. There were also significant changes in the X-ray absorption ratio in the heads. The accumulation of ⁹⁹mTc-MDP was higher in all long-bone heads after hyperbaric exposure than before exposure. These results suggest that the hyperbaric environment promotes bone calcification.


Keywords: hyperbaric exposure, Digital Magnification Radiography, bone

Introduction

During long-term space flight, astronauts are known to have a normal serum concentration of calcium, while excreting increased amount of calcium in the urine. It has been reported that once decalcification occurs, the bone cannot be completely recovered even after five years and that the density of astronaut's bones is lower after space flight than before it (Kashima, 1992). To prevent decalcification and increased calcium excretion, astronauts regularly exercise in a spacecraft and wear pressure-loading spacesuits (Sekiguchi, 1988). I wondered if the atmospheric pressure was a factor in this phenomenon and, if so, could the reverse be true.

The purpose of this study was to evaluate the effect of hyperbaric pressure on bone calcification, and if a positive relationship existed, to consider the application of pressure in treating osteoporosis. The analysis of bone-mineral content with serological, biochemical, and histological examinations, are highly reliable but involve technical problems (Matsumoto and Nakamura, 1995). Conventional X-ray examinations do not allow the detection of changes of less than 30-40% on X-ray films. In previous studies (Nishimura, 1993; Nishimura et al., 1996), we developed digital magnification radiography (DMR), which allows visual detection of bone changes, by combining an imaging plate (IP) using a photo-stimulable phosphor, an X-ray imaging sensor, and computed radiography (CR). In addition, we could quantitatively analyze changes in bone-mineral content by determining changes in the X-ray absorption ratio using a bio-imaging analyzer (BAS), which is frequently used in the field of molecular biology.

In this study, changes in the heads of long bones under hyperbaric conditions were examined by image technology to substantiate the hypothesis that bone calcification is promoted by controlling pressure.

Materials and Methods

Experimental protocol and hyperbaric exposure

Thirty male Wister mice (13 weeks old), weighing 35-40 g were subjected to 1 atm abs in air for two weeks. This period was sufficient the animal to adapt to the pressure chamber. Twenty-five mice were then subjected to 2 atm abs (200 kpa) with mixed gas (He-O₂: 88%:12%) for 2 weeks. This pressure is equivalent to that in 10 meters of sea water. The compression rate was 5 m/min and decompression was non-stop, but less than 0.5 m/min. The temperature in the pressure chamber was maintained at approximately 26-28°C, and a relative humidity of 60%. Thirty mice for the Sham control group were also placed in a pressure chamber at 1 atm abs with air for the same period as the pressure group. These methods minimized measurement errors caused by widely varying body weight (Fig. 1). Analysis was performed at six stages: before acclimation, before increasing pressure, and immediately, 24 hours, one week and two weeks after decreasing pressure (Fig. 2).

Digital magnification radiography

Regions of interest (ROI) were the heads of the humeri, femora, and tibiae at each stage. Mice were treated with Nembutal at 10 mg/kg i.p. prior to extracting these bones.
To obtain clear images of the extracted bones, 10-fold directly magnification radiography was performed, using a highly sensitive imaging plate (IP: Fuji Photo Film Co.; Tokyo Japan. ST type) combined with a micro-focus tube (MFT: Pony Atomic Industry Co.; Tokyo Japan P70-III) with a focus size of 10 × 10 μm. During X-ray exposure, the conditions were: tube voltage: 30 kVp, tube current: 40 μA, focus object-distance (FOD): 25 mm, focus IP-distance (FIPD): 250 mm, and exposure time: 15 seconds. The images obtained were magnified two fold by computed radiography (CR: Fuji Photo Film Co.; Tokyo Japan, CR AC-1 Plus) to produce digital magnification radiograms (DMR) that were enlarged 20-fold (Nishimura, 1993). Ten doctors visually evaluated the obtained DMRs using a ranking scale from 1 to 5 (5: clearly more dense, 4: possibly more dense, 3: unchanged, 2: possibly less dense, and 1: clearly less dense compared to the control images). DMR images of the controls were obtained at 13 weeks.

**Quantitative analysis**

To conduct a quantitative analysis, as with DMR, 10-fold magnification radiography was directly performed on each bone using IP (Fuji Photo Film Co.; Tokyo Japan, UR type) and MFT. During X-ray exposure the conditions were: tube voltage: 25 kVp, tube current: 40 μA, FOD: 25 mm, FIPD: 250 mm, and exposure time: 15 seconds. On the radiographic images obtained, photo-stimulated luminescence (PSL) representing the number of transmitted photons was measured using a BAS with a reading pixel size of 50 × 50 μm (BAS-3000: Fuji Photo Film Co., Tokyo Japan) to determine the X-ray absorption ratio. The block diagram for analysis is shown in Fig. 3. Quantitative analysis was

**Fig. 2** Experimental protocol. Before hyperbaric exposure, the mice were housed in a experimental chamber for small animals for two weeks at normal atmospheric pressure to prevent the loss of body weight due to the stress of being locked up in the small chamber. All animals were then subjected to hyperbaric exposure for two weeks at a pressure of 2 atm abs (200 kpa), a PO₄ of 0.31 bar, a temperature of 26–28°C, and a relative humidity of 60%.

**Fig. 3** Block diagram. The system consists of an Imaging plate (IP), Micro-Focus Tube (MFT), Bio-imaging analyzer (BAS), and Computed radiography (CR:AC-1).
performed in all areas of the heads of the humeri, femora and tibiae. In addition, the thickness of the bone head was measured to examine the effects of the growth and development of the bone at each stage. The significance of difference was evaluated by the Student’s t-test.

Accumulation of $^{99m}$Tc-methylene disphosphonate ($^{99m}$Tc-MDP) into bone tissue

To examine the uptake of $^{99m}$Tc-MDP per gram weight, mice were treated with $^{99m}$Tc-MDP at 1.27 MBq/g i.p. before and after hyperbaric exposure. After three hours, the bones were resected and subjected to three-hour contact exposure to a UR-type IP. The exposed IP was analyzed with a BAS to obtain autoradiographs for visual detection. Then, the uptake of $^{99m}$Tc-MDP was determined using a Aloka scintillation counter (Auto Well Gamma System:ARC-500).

Results

Figure 4 shows the DMR images of the heads of long bones (the humeri, femora, and tibiae) in the exposure group (at the ages of 13, 15, 17, 18, and 19 weeks). DMR techniques could provide clear enough images of the heads to enable visual evaluation. The images clearly showed radiolucency, suggesting increased calcification at two stages, 24 hours and one week after hyperbaric exposure, in all cases.

Figure 5 shows the results of a visual evaluation of DMR images in Fig. 4 by 10 doctors. The vertical and horizontal
axes represent five ranks for the evaluation and stages, respectively. The bones were judged to be more compact at 17 weeks (post exposure 24 hours) and one week after hyperbaric exposure.

Figure 6-a, b, and c show changes in the X-ray absorption ratio in the control and exposure groups. In the tibiae (a), the absorption ratio was highest at 19 weeks, being 1.7% higher in the exposure group than in the control group ($P<0.025$). In the humeri (b), the maximum absorption ratio observed (24 hours after end of hyperbaric exposure) was 3.09% higher than that of the control group ($P<0.005$). In the femora (c), the maximum absorption ratio observed 24 hours after hyperbaric exposure was 3.75% higher than the corresponding control value ($P<0.005$).

Figure 7 shows autoradiographs obtained using $^{99m}$Tc-MDP. The accumulation of $^{99m}$Tc-MDP was higher in the heads of long bones after hyperbaric exposure than...
morphological abnormalities in the bone can be detected but it is difficult to visually grasp changes in the microstructure. It is also difficult to quantify the mass of bone-mineral through digitalized radiographs. Two methods are available for the digitalization of radiographs. The first is the use of film itself as an indicator of density. Another method requires devising an X-ray generator and detector, to show the absorption of X-rays at each measured site. With the former method, using X-ray films, it is said that there are difficulties in adjusting many factors such as the measurement and development conditions (Matsumoto and Nakamura, 1986; Noda and Sato, 1987).

Cameron and Sorenson (1963) reported that the content of bone-mineral can be quantified in vivo using the transmittance of photon beams (Sorenson and Cameron, 1967). In Japan, Orishige first applied a bone-mineral analyzer (Norland Cameron Co., Ltd), to the treatment and evaluation of bones (Orishige, 1980). With this method, however there are problems with reproducibility and limited measurement sites.

In the present study, we used an X-ray image sensor with a photo-stimulable phosphor. Radiography was performed using an IP and an X-ray generator MFT with a small focus size. From the radiographic images obtained, DMR was performed using a CR with a pixel size of 100 × 100 μm (widely used in clinical practice in Japan). Quantitative analysis was performed using a BAS with a reading pixel size of 50 × 50 μm. This method allowed us to visually evaluate the changes in the microstructure of the heads of long bones (humeri, femora, and tibiae) in mice.

Using quantitative analysis based on changes in the absorption ratio, we determined the change in calcium content per unit area instead of per unit volume. Thus, our results would include increased calcium from both increased bone density and increased bone thickness. Accordingly, we could not determine whether the change in the absorption ratio was due to a change in calcium density or a change in thickness caused by the growth and development of the bone. However, since the mice used in this study were aged 13 weeks or more, the growth and development of the bone is expected to be almost completed (Tajima, 1991). Also in our study, the standard deviation of the rate of the change in bone thickness at that age is ± 0.02% for the humeri, ± 0.01% for the femora, and ± 0.03% for the tibiae, all of which are negligible. Therefore, the increase in the X-ray absorption ratio may represent increased calcium density rather than increased thickness.

The bone consists of two parts, highly dense cortex and less dense spongion. Generally, the thickness and density are evaluated for the cortical bone and the configuration of bone trabeculer for the spongy bone. In the DMR image obtained in this study, there was

Figure 8 shows the uptake of $^{99m}$Tc-MDP per gram weight. In the humeri, the uptake of $^{99m}$Tc-MDP per gram weight was increased by 2.63 %/g by hyperbaric exposure (P<0.025). In the femora, the uptake of $^{99m}$Tc-MDP per gram weight was 2.04% /g higher than the before hyperbaric exposure (P<0.01). In the tibiae, the uptake of $^{99m}$Tc-MDP per gram weight was 1.32 %/g higher than the before hyperbaric exposure (P<0.01).

Discussion

There have been various reports on bone changes due to hyperbaric exposure. These describe dysbaric osteonecrosis (DON), decompression sickness (DCS), and hyperbaric oxygen therapy (HBO which promotes bone growth by stimulating collagen synthesis), (Criswell and Mehm, 1992; Davis et al., 1990; Lin et al., 1993; Lehner et al., 1993; Rambaut and Johnson, 1979; Watson et al., 1975).

The bone functions as a supporting structure and plays an important role in dealing with gravity. The bone is continuously remodeled to maintain an optimal structure and function against external stresses. This means that the composition and shape of the bone are always changing. Thus, it is important to check bone changes and the degree of change as surrounding conditions change.

Currently, the composition and shape of the bone are evaluated by serological and X-ray examinations and analysis of histological parameters for hard tissues, but in many cases the changes cannot be detected at an early stage. Analysis of histological parameters for hard tissues is highly reliable, but there are some problems in the preparation of specimens. In X-ray examination,
radiolucency suggesting increased calcification in all bones examined after hyperbaric exposure. This finding is consistent with the results of experiments with $^{99m}Tc$-MDP.

Human bone always change its shape to support the optimal load. This is known as Wolff's theory (Wolff, 1986).

Kashima (1992) reported that divers with 500 hours or more of diving time showed increased density in the mandible as imaged by CR.

The results of the present study suggest that pressure loading increases mechanical stress on the bone, resulting in increased bone density. Based on this characteristic of bone, we can promote calcification by carefully controlled hyperbaric exposure.

In this study, Heliox gas was used for the atmospheric pressure. The reason is that this study investigates the condition of the bone without oxygen tension influence. In future, the influence of different kinds of gas on the bone, such as Nitrox and air and not only Heliox will be examined.

Conclusion

The effects of hyperbaric exposure on long bone heads in mice were investigated by imaging technology, and obtained the following results.

1. Using the combination of MFT, BAS, and CR, we depicted hyperbaric exposure-induced changes in the heads of humeri, femora, and tibiae at each stages, then performed quantification based on changes in the X-ray absorption ratio.

2. In DMR images, there was enhanced opacification suggesting increased calcification 24 hours and one week after hyperbaric exposure.

3. The exposure group had a significantly higher absorption ratio than the control group.

4. In all of the long bones tested, the uptake of $^{99m}Tc$-MDP per gram weight was increased after hyperbaric exposure.

5. Autoradiographs also showed increased accumulation of $^{99m}Tc$-MDP in long bones after hyperbaric exposure. These results suggest that bone calcification can be promoted by physical pressure.

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