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The Effect of Calcitonin With Physical Training to Increase Bone Density

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ABSTRACT
The aim of this study is to determine the influence of a combination of calcitonin with physical training to increase bone density. The method used is randomize posttest only control group design. The group consists of the control group, physical training group and the combination group. The samples of this research were seven young norvegicus rats (six weeks old). Physical training group does swimming once a day with a frequency of three times a week, the control group was given injections of calcitonin salmon calcitonin synthetic 2 IU/100gram weight every day and the combination group were given physical training and hormone calcitonin injection. The duration of treatment was eight weeks. Bone density measurement was done using ultrasound Digital Bone Measurement-sensor (DBM) SONIC 1200. These instruments emit sound waves to measure bone density with m/sec. Based on the data analysis described the combination hormone calcitonin administration and physical training have higher bone density (p=0.001) compared with the other groups. In conclusion, there is the combined effect of the hormone calcitonin and physical training on bone density improvement.

Keywords: *Calcitonin, bone density*

1. INTRODUCTION

Osteoporosis is one form of bone abnormality characterized by decreased overall bone mass accompanied by damage to bone architecture resulting in decreased bone strength. In Indonesia the number of people with osteoporosis continues to increase. This can be seen with the increasing incidence of femoral fractures resulting from osteoporosis in 2007-2010, from about 20 thousand cases in 2007 increased to about 43 thousand cases in 2010 (MOH, 2010). WHO records about 200 million people suffering hip fractures due to osteoporosis worldwide (WHO, 2012).

Bone is a complex network consisting of cells and matrices. Bone matrix is formed by fibers and basic substances containing mineral salts. Mass and bone thickness at all times always experience the dynamics of addition and subtraction through remodeling process (bone matrix is absorbed and formed again). Remodeling aims to keep bones in shape and structure. The cells that play the role of bone are osteoblasts and the cells that play bone-absorbing are osteoclasts. Normal osteoblasts are able to put collagen type I and form new bone, while osteoclasts erode and absorb bone that has been formed. The process of formation and absorption of bones it is necessary efforts to maintain bone density from an early age. Having a high bone mass means strong and healthy bones so it is not easily kropos and fragile to avoid early osteoporosis (Junqueira, 2007; Ganong, 2008).

One effort that can be done to increase bone thickness of epiphyses and bone density is by physical exercise. From several studies, physical exercise performed regularly and with certain doses leads to an increase in bone density, bone size and bone shape (Ide, 2012). Physical exercise is one of the physical stressors that can affect bone composition. Physical exercise is a recurring physical activity and aims to maintain, improve and express fitness. Physical training is very diverse types such as brisk walking, jogging, cycling, swimming and all types of gymnastics (Bompa, 1994).

In physical exercise there is an exercise component consisting of dose of exercise (duration, distance and number of reps), intensity (load and speed) and frequency of exercise. The intensity of the exercise shows the qualitative components of work performance performed during a particular time exercise. The degree of intensity can be measured by type of sport (Bompa, 1994) . Several studies were conducted to determine the effect of type of exercise intensity on bone density. Research conducted by running on a treadmill for 30 minutes (moderate intensity) and 60 minutes (high intensity) at a speed of 45 cm/s in 60-day-old male rats found a significant increase in left and right tibial bone density in the 30-minute span group. The study concluded that moderate-intensity exercise was excellent for the development of bone mass (Ertem *et al* , 2008).

There are several hormones that can affect bone tissue such as calcitonin, parathyroid, growth hormone, androgens and estrogens (Junqueira, 2007). Calcitonin is a polypeptide hormone that plays a role in lowering plasma calcium. In its development was created a synthetic or recombinant of different species of calcitonin pigs, calcitonin eels and calcitonin salmon that have been used for medical purposes. Calcitonin salmon is by far the most commonly used in clinical practice because it is 40-50 times higher in intrinsic potential when compared with human calcitonin, and its better analgesic properties (Ganong, 2008). In humans calcitonin is usually used to treat postmenopausal disease, osteoporosis , paget bone disease , and hypercalcemia. The role of salmon calcitonin in normal human bone physiology is still not clearly understood (Novartis, 2009). The effects of salmon calcitonin on density of tulan g are less clear than with other antiresorptive administration, especially bisphosphonates and *strontium ranelates* . Its ability to prevent vertebral fractures has been demonstrated in clinical studies in postmenopausal women with 200 IU/day salmon *nasal spray* calcitonin reducing the risk of fractures by 36%. It seems to be mediated through the formation of bone microarchitecture and decreased bone resorption. Bone density increased over five years by about 1.5% when compared with baseline measurements and increased by 1% compared to the placebo group. It can be concluded that salmon calcitonin has only moderate effects on bone density (Chesnut et al, 2008)

The purpose of this study to determine the potential for salmon calcitonin and physical exercise in n kapadatan raise the bone, from the research results expected to be an advanced alternative to early osteoporosis prevention efforts so that the incidence of osteoporosis in Indonesia can be reduced.

2. METHOD

The research is experimental research using the posttest-Only Control Group Design design. The sample of the study was white rats (*rattus norvegicus*) of the growing period of age, weighing 160-180 grams, the physical health condition, obtained from the animal research unit of the Biochemical Laboratory of the Faculty of Medicine, Airlangga University. Sampling was performed on mice aged six to eight weeks of age which is the age of early puberty in mice (Kusumawati, 2004). Taking rats at growth period according to Mackovic (1994) 90% bone mass formed at the age of 12-14 years at prepubertas and puberty (Yuliati, 2002). Peak bone period is reached when a person is around 30 years old and after that bone mass will be reduced due to the start of the imbalance of remodeling process (Ide, 2012). Male sex was chosen for sample homogeneity.

Sampling technique in this research is done by simple random sampling. Twenty-four rats were randomly divided into four groups, each group consisting of six rats, a control group, calcitonin , physical exercise groups , and combination groups .

The tools used in this research are *Ultrasound DBM Sonic 1200*, torbal scales, bucket for swimming with diameter 50 cm, height 60 cm with water depth 40 cm, water temperature 30 - 36 ^{0} C, stopwatch to calculate the length of pool time, injection. The ingredients used in this study were *Ketamine HCL*, salon calcitonin reponent, KY lubricatting jelly.

Giving calcitonin salmon obtained from preparations *Miacalcin* the form of a solution with a dose 2UI/100 gr weight rat/day in experimental animals group calcitonin and combinations, are given by way of injected per subcutaneously once daily for eight weeks provided in the morning and for the combination group given after physical exercise. Dose calculation based on previous research (Khaldi *et al*, 2005).

Provision of physical exercise by calculating the maximum time swimming ability first. Maximum time is obtained by the way each mouse swims up to the sinking of a mouse marked with the first big air bubble. The training time of each rat was 85% of the maximum time achieved by each mouse. Exercise given once a day, with a frequency of three times a week on Monday, Wednesday and Friday for 8 weeks (Bompa, 1994; Kragel *et al*, 2006; Coutinho *et al*, 2011). After eight weeks' treatment the bone density of the mice was measured using a *Ultrasound DBM* with a measurement unit m/sec, which measured the metaphysical bone of the femur.

Bone density measurements were performed using *Ultrasound DBM*. This tool produces a conduction of sound waves that assess the bone density with the m/s unit. Human examination using the *Ultrasound DBM* was performed on the metaphysical bone of the proximal phalanx of the fingers as it provides a good indication of mineralization and the condition of bone tissue structure (Dalen, 1993). Macroscopic observation of bone consists of cortical (compacta) and trabecular (spongiosa) bone. Trabecular and cortical bones are present in every bone but their numbers and distribution are very different. Trabecular bone is more likely to undergo mineral changes, therefore the surface is larger. In long bones the metaphysical area has more trabecular bone tissue than the cortical bone (Borer, 2005). The speed of sound waves will be faster on solids and liquids. The speed of sound waves in water is 4 , 3 times the speed in the air, which is 1,484 m/sec . The speed of sound in iron is 15 times the speed in the air, which is 5.120 m/sec. It can be concluded that the more density the object the faster the sound wave, so the higher the measurement results or the faster the sound wave sounding in the bone network means that the measured bone is more solid.

In this study it is not possible to measure the proximal mouse phosphate because the size of the bone is very small to be measured by placing the *probe* (the device where the sound waves) *Ultrasound DBM*. Based on the previous study selected animal legs try in the metafisis femur rat as a measurement area with consideration the similarity of anatomy and physiology. In addition, the selection of the metaphysical area is based on the ease of placement of the *probe* so that it can be measured appropriately.

Treatment of the study was conducted for eight weeks based on the time required for osteoblasts to fill the recessed cavity (Raisz, 1988 *cit* Sunoto, 2001). The research was conducted at Animal Try Unit of Biochemistry Laboratory of Medical Faculty of Airlangga University.

Data were tabulated and analyzed statistically through the following phases: to test the normality of the data by the *One-Sample Kolmogorov-Smirnov Test,* test of homogeneity of variance using ANOVA, provided the data were normally distributed with variance homogeneous, if the obtained results of the analysis of H_0 is rejected, then proceed with *Least Significant Difference* (LSD) test to know the difference between treatments.

3. RESULTS

In accordance with the design of data analysis, the initial step of the analysis will begin by conducting descriptive statistical analysis aimed at obtaining a description of the distribution and for data summary for the presentation of results.

4. CONCLUSION

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Table 1. Descriptive data on bone epidatics

Group		Bone density (m / s)
Control	average	1572.14
	SD	38.17
physical training	average	1625.14
	SD	57.07
Combination	average	1701.57
	SD	99.42

The next data is analyzed by different test using anova test which aims to see the effect of intergroup treatment on dependent variable.

Table 2. Anova test results on bone density variables

Variables	F	p
Bone density	4, 601	0.011

Statistical test results *One Wa y ANOVA* showed that bone density has a value of p = 0.0 11 (α = 0.05) and severe bone has a value of p = 0.032. The p value <0.05 indicates that there is a significant difference for the mean bone density in the whole group. To determine which groups differed in bone density and bone density variables, the analysis continued with *Post Hoc Tests* with LSD.

Table 3. *Post Hoc Test* Result on variable bone density

Group		Mean difference of bone density	p Bone density
Control	physical training	5.300.000	0.147
	Combination	12,942,857	0.001
physical training	Combination	7,642,857	0.041

Result of LSD test (p-comparison comparison) variable of bone density was significant difference between control group and combination group p=0,001 (p<0,05), between physical exercise group with combination p = 0,041 (p<0,05). On variable of bone weight difference was significant between control group and combination p = 0,004 (p<0,05).

These results can be explained based on bone remodeling process. bone has its addition and mineral reduction through bone remodeling mechanism. The remodeling process involves the process of bone resorption by osteoclast cells and bone matrix formation by osteoblast cells (Ganong, 2008). Calcitone plays a role in inhibiting the activity and formation of osteoclast cells. This is consistent with the Naot and Cornish (2008) statements which suggest that the target cell of calcitonin lies in osteoclast cells and plays a role in influencing bone resorption processes. The statement is also supported by Zaidi, Moonga and Abe (2002) which states Both ends of the calcitonin molecule contain species that are residual invariant needed to bind G-proteins that are receptors on osteoclasts. Calcitonin inhibits osteoclast motility, causing immobility through the cAMP mechanism. The ligand specificity of the calcitonin receptor isoform leads to protein-modifying receptors (RAMPs) that form heterodimers that may play a role in inhibiting the osteoclastogenesis process (Zaidi *et al*. 2002; Naot *et al.*, 2008) .

Physical exercise affects changes in bone metabolism according to Ocarino (2006) through direct effect and indirect effect, direct effect through mechanical force while indirect through hormonal factors. Mechanical strength when applied in bone tissue forms an endogenous sign. The signs are captured by the *mechanosensoric* system to be captured by the osteocytes and then turn them into biochemical signs that govern bone turnover. Mechanical forces stimulate the release of *prostaglandin E2* (PGE2) from the gap junction. PGE2 binds to receptors in osteocytes and stimulates the formation of bone matrix proteins. Indirect effects of physical exercise affect bone metabolism is to stimulate the secretion of the growth hormone. Physical exercise will cause a stressor that will stimulate the anterior pituitary secreting growth hormone. Growth hormone will stimulate the liver to produce *Insulin-Like Growth Factor-1* (IGF-1) which will improve the performance of osteoblast cells (Guyton 2006; Ocarino, 2006).

From the description above there appears sinergitas calcitonin performance and physical exercise of bone remodeling process. Decreased performance and bone formation caused by salmon calcitonin and increased osteoblast performance caused by physical exercise cause the bone matrix formation process to increase while bone resorption decreases. Such a remodeling mechanism will cause the bone matrix to increase so that bone density will increase.

Looking at the results of descriptive statistic research there are differences in the mean in each group of each bell. Post Hoc Test results also showed no significant difference between the control group with calcitonin and between the dick group and the physical exercise group on bone density variables and bone weight variables (p>0.05). This result seems to be affected by the minimal amount of data variation, one treatment group consisting of only 7 samples.

This study aims to prove the administration of salmon calcitonin and physical exercise will increase bone density during growth. From birth bone mass grows to peak at about 30 years of age. After the age of 30 years bone mass will decrease gradually. In the elderly it can be avoided from early osteoporosis if it can keep the bones remain strong while young. Having a high bone mass means strong and healthy bones so it is not easy to fragile. Increasing bone density during the growth period is expected bone density will be optimal when the growth period ends so as to avoid early osteoporosis. The results concluded that calcitonin and physical exercise increased bone density of growth-expanding white rats. The combination of calcitonin and physical exercise had a significant effect on bone density of growth-expanding white mice.

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