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by Gadis Meinar Sari

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Mechanism of Bone Metabolism Interruption Due to High Intensity Physical Exercise

Gadis Meinar Sari^{1*}, Soetjipto², Lilik Herawati³

¹Department of Physiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Department of Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

³Sport Health Magister Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Corresponding Author: Gadis Meinar Sari

Email: gadis-m-s@fk.unair.ac.id

ABSTRACT

Background: High-intensity physical exercise affects the increase of cortisol secretion which knows as the main glucocorticoid in the body and causes a decrease in osteoprotegerin (OPG) activity. Glucocorticoids also work directly on osteoblasts and osteocytes through glucocorticoid receptors (GR α).

Aims: this study aimed to prove the mechanism of bone metabolism disorders due to high-intensity physical exercise through changes in glucocorticoid levels, osteoprotegerin, osteocalcin, c-telopeptide and the number of apoptotic osteoblasts.

Method: an experimental study with a quasi-experimental design was used. Object used was 20 female white rats of approximately 3 months of age divided into the control and treatment groups. In the treatment group was given swimming training with 18% weight gain within 90% of the maximum time, carried out 2 times per set with a frequency of 3 times per week within 8 weeks. The data obtained were analyzed by t-test 2 independent samples and path analysis.

Result: The results of analysis showed an effect of high-intensity physical exercise on glucocorticoid levels ($r=0.79$); glucocorticoid levels affected the level of osteoprotegerin ($r=-0.688$) and osteocalcin ($\beta=-0.48$), however it did not affect the number of apoptotic osteoblasts ($r=0.21$); osteoprotegerin levels affected c-telopeptide levels ($\beta=-0.65$); the number of apoptotic osteoblasts had no affected on osteocalcin levels ($\beta=-0.19$); levels of c-telopeptide ($\beta=0.82$) and osteocalcin ($\beta=-0.22$) affect the c-telopeptide or osteocalcin ratio.

Conclusion: The mechanism of bone metabolism on high-intensity physical exercise is caused by the increase of glucocorticoid levels which makes a decrease in osteoprotegerin levels and affects on the rise of c-telopeptide levels.

Keywords: High-intensity physical exercise, glucocorticoids, osteoprotegerin, osteocalcin, c-telopeptide, apoptotic osteoblasts, bone remodeling.

Correspondence:

Gadis Meinar Sari

Department of Physiology Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Email: gadis-m-s@fk.unair.ac.id

INTRODUCTION

The body has the ability to respond and adapt to increasing doses of physical exercise, including frequency, intensity, time and form of exercise. Giving a dose of exercise which exceeds the threshold value of the body's adaptability can be a stressor that has a negative impact on the body[1, 2]. A study states that high-intensity physical exercise affects the increase of cortisol secretion which knows as the main glucocorticoid in the body. Research on athletes who ran on a treadmill for three hours at speeds like when running a race showed a 60% increase in the hormone cortisol. High glucocorticoid levels can disrupt the balance of bodily functions so that it has pathological effects, one of which is on bones[1-3].

While osteoporosis is a bone remodeling disorder that often occurs, it is a bone disorder caused by an imbalance in the formation and resorption process that resulting in decreased bone density and changes in the micro structure of bone architecture so the bones become brittle and increase the risk of fractures.[3, 4] Osteoporosis ranks as a major disease problem in 10 million people over 50 years.[5] Globally, osteoporotic fractures caused an estimated 5.8 million disabilities in 2000 which were also related to increased mortality.[6] In the last 30 years in Asia, there has been an 2-3 fold increase in the incidence of osteoporotic femoral fractures. It is estimated that in 2050 osteoporotic

femoral fractures in the world reach 6.26 million people and in Asia 3.25 million (52%).[7]

Throughout life, the bone will undergo a process of remodeling, which is an interconnected mechanism between formation by osteoblasts and resorption by osteoclasts. In the formation process, osteoblasts produce a number of proteins including osteocalcin which are non-collagen proteins which can be used as biochemical markers of bone formation processes.[8-11] At the time of bone resorption, osteoclasts release the result of degradation of type one collagen protein matrix including cross-linked telopeptide (C-telopeptide) which can be used as biochemical markers of bone resorption processes.[8, 12, 13]

Decreased in bone density occurs due to an imbalance in bone remodeling process in which the resorption process of osteoclasts is not followed by the formation of osteoblasts. The continuation of osteoclastogenesis was due to the binding of the receptor activator of nuclear factor- κ B (NF- κ B) or RANK expressed in osteoclast progenitors, macrophage hematopoietic cells and monocytes with RANK-ligand (RANKL) expressed in preosteoblastic cells, while T lymphocytes The binding of RANK and RANKL will stimulate the differentiation of osteoclast and activation of osteoclast progenitors to active osteoclasts so that there is an increase in bone resorption process.[11, 14] Glucocorticoids cause a decrease in osteoprotegerin (OPG) activity, a soluble

decoy receptor released from stromal cells or osteoblasts which has a binding effect on RANKL so that it can inhibit RANKL-RANK interactions.[15-21] On the other hand glucocorticoids also work directly on osteoblasts and osteocytes through glucocorticoid receptors (GR α) on bone forming surfaces, where remodeling processes occur.[22] The activity of intracellular kinase Proline-rich tyrosine kinase 2 (Pyk2), which influences cytoskeleton reorganization and apoptosis, will be modulated by glucocorticoids. Glucocorticoids affect the entry of extracellular Ca² resulting in an increase in intracellular Ca² which will accelerate the process of Pyk2 phosphorylation. Pyk2 activation in turn activates c-Jun N-terminal kinase (JNK) which causes osteoblast apoptosis.[6, 23-25]

METHODS

This study aims to explain the mechanism of bone metabolism disorders due to high-intensity physical exercise through changes in glucocorticoid levels, osteoprotegerin, osteocalcin, c-telopeptide and the number of apoptotic osteoblasts. This study was an experimental study using a quasi-experimental design. The period of research was from January to March 2017.

Sample

The samples case were 20 female white rats (*Rattus norvegicus* strain Wistar) which were approximately 3 months old that were divided into two groups, the control and treatment groups. In the treatment group given high-intensity physical exercise in the form of swimming training with 18% weight gain within 90% of the maximum time, carried out 2 times per set with a frequency of 3 times per week, for 8 weeks. At the end of the treatment, glucocorticoid, osteoprotegerin, osteocalcin, c-telopeptide levels were examined and apoptotic osteoblast counts were measured by examining 5 ml blood of the rats in treatment group which collected through intracardial. Whereas, in the control group had no given treatment. The research was carried out at several Laboratories of Universitas Airlangga, Surabaya, Indonesia.

Procedure

The treatment of high-intensity physical exercise was carried out by swimming the mice by loading 18% body weight with 90% of the maximum time (T90) at intervals of 2 times, resting time between 2 intervals was 2 times of T90. Won with a frequency of 3 times per week for 8 weeks[26, 27]. Every week weighing is done to adjust the weight by 18% body weight. After 8 weeks of treatment, animals were anesthetized with ether after 15 minutes of treatment[28]. Intracardial blood samples were taken approximately 5 ml and put in a vacuum tube without coagulants for examination of glucocorticoid, osteoprotegerin, c-telopeptide and osteocalcin levels. Try animals were sacrificed by increasing the dose of ether, to be taken part of the upper limb (femur) and cleaned/removed muscle tissue attached to the bone. Decalcification of femur bone to remove calcium in bone tissue so that the preparation is easily sliced for microscopic preparation[29]. Immunohistochemical preparations for microscopic examination are made to count the number of osteoblasts undergoing apoptosis.

Data Analysis

The data obtained were analyzed by t-test 2 independent samples (independent t-test) and path analysis. Statistical analysis was found to be significant if the number of p-values reach about <0.05. The data collected was processed by using SPSS program version 17. (SPSS, Inc. Chicago, IL)

RESULT

Blood glucocorticoid levels

The average glucocorticoid levels in the treatment group were higher than in the control group while the variation of the data was almost the same in the two groups. T-test results of free samples showed that there were significant differences between the treatment and control groups ($p < 0.05$). The minimum value of glucocorticoid levels in the treatment group was greater than the control group. While the minimum price of glucocorticoid levels in the treatment group was close to the maximum price of the control group. (Table 1)

Table 1 Descriptive Data of Variable Analyzing

Variables	Group	\bar{x}	SD	Min	Max	Independent t-test
Glucocorticoid (ng/ml)	Treatment	9.63	2.84	6.10	13.56	t=-5.39 p=0.000*
	Control	3.29	2.39	0.36	8.50	
Osteoprotegerin (pg/ml)	Treatment	40.24	13.88	13.70	53.70	t=9.04 p=0.000*
	Control	104.40	17.63	80.50	140.10	
Osteocalcin (ng/ml)	Treatment	82.99	14.40	50.20	99.00	t=4.63 p=0.001*
	Control	162.21	52.14	99.00	283.20	
Apoptosis Osteoblast (cell/lp)	Treatment	18.40	20.22	0.00	62.00	t=-1.78 p=0.101
	Control	6.30	7.12	0.00	21.00	
C Telo peptide (ng/ml)	Treatment	5.37	2.71	2.41	12.52	t=-4.05 p=0.001*
	Control	1.82	0.51	0.63	2.51	
Ratio of C-Telo peptide/Osteocalcin	Treatment	0.07	0.03	0.03	0.14	t=-5.54 p=0.000*
	Control	0.01	0.00	0.00	0.02	

Legend: * significant on $\alpha = 0.05$

Blood osteoprotegerin levels

The mean osteoprotegerin level in the treatment group was lower than in the control group and the variation of the data was almost the same in the two groups. T-test results of free samples showed that there were significant differences between the treatment and

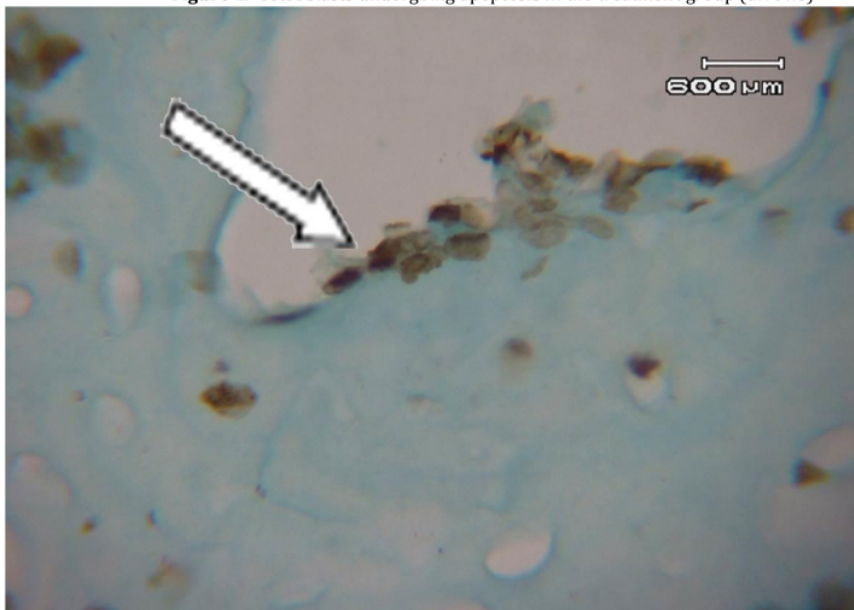
control groups ($p < 0.05$). The minimum value of osteoprotegerin levels in the treatment group was lower than the control group. The maximum price of the treatment group was much lower than the minimum price of the control group. (Table 1)

The number of apoptotic osteoblasts in bone tissue

The mean number of apoptotic osteoblasts in the treatment group was higher than in the control group and the variation of data in the treatment group was very high. T-test results of 2 free samples showed no difference between the treatment and control groups ($p > 0.05$). The maximum value of the number of apoptotic osteoblasts in the treatment group was higher than the control group. (Table 1)

The results of osteoblasts undergoing apoptosis by the Tunnel assay method with 400 x magnification. The method used in this examination is a quantitative method, where the number of apoptotic osteoblasts in each sample is determined by adding all apoptotic osteoblasts found in five different fields of view, at 400 times magnification. In this examination osteoblast cells undergoing apoptosis show dark brown to black color in the cell nucleus that indicates the occurrence of DNA fragmentation. (Figure 1)

Figure 1: osteoblasts undergoing apoptosis in the treatment group (arrows)



Blood osteocalcin levels

The mean levels of osteocalcin in the treatment group were lower than in the control group and the variation of the data was almost the same in both groups. T-test results of free samples showed that there were significant differences between the treatment and control groups ($p < 0.05$). The minimal value of osteocalcin levels in the treatment group was lower than the control group. The maximum value of osteocalcin levels in the treatment group was the same as the minimum price of the control group. (Table 1)

Blood c-telopeptide levels

The mean c-telopeptide level in the treatment group was higher than in the control group and the variation of the data was almost the same in the two groups. T-test results of free samples showed that there were significant differences between the treatment and control groups ($p < 0.05$). The maximum value of C-telopeptide levels in the treatment group was greater than the control group. (Table 1)

The ratio of blood c-telopeptide / osteocalcin

The mean C-telopeptide/Osteocalcin ratio in the treatment group was higher than in the control group and the variation of the data was almost the same in the two groups. T-test results of free samples showed that there were significant differences between the treatment and control groups ($p < 0.05$). The minimum value of the C-Telopeptide or Osteocalcin ratio in the treatment group was greater than the control group. The maximum price of the control group was close to the minimum price of the treatment group. (Table 1)

The effect of high-intensity physical exercise on glucocorticoid levels

The results of categorical regression analysis showed that there was an effect of high-intensity physical exercise on Glucocorticoid levels ($p < 0.05$). (Table 2) The analysis showed that the positive path of coefficient means that if high-intensity physical exercise increases, glucocorticoid levels increase as well ($\gamma = 0.793$).

Table 2. Coefficient Value of high-intensity physical exercise on glucocorticoid levels

	Standardized Coefficients		df	F	Sig.
	γ	bootstrap (1000) Estimate of Std. Error			
Physical Exercise of Treatment Group	0.79	0.07	1	109.93	0.000

Dependent Variable: Glucocorticoid

(*) Significant results with p -value < 0.05

γ : symbol for the path from independent to dependent variable

The effect of glucocorticoid levels on osteoprotegerin and apoptotic osteoblasts

The results of the regression analysis showed that there was a change in glucocorticoid levels on Osteoprotegerin ($p < 0.05$). (Table 3) The results of the analysis show a negative path coefficient, that shows the opposite change, if Glucocorticoid levels increase then Osteoprotegerin levels will decrease ($\gamma = -0.678$).

Regression analysis showed that there was no effect of changes in glucocorticoid levels on the number of apoptotic osteoblasts ($p > 0.05$). (Table 2) The analysis showed a positive path coefficient, meaning that if there is an increase in glucocorticoid levels, the number of Apoptotic Osteoblasts will increase ($\gamma = 0.212$).

Table 3. Coefficient value of glucocorticoid and apoptotic regression levels

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error			
(Constant)	111.35	11.44		9.73	0.000
Glucocorticoid	-6.04	1.50	-0.68	-4.02	0.001
a. Dependent Variable: Osteoprotegerin					
(Constant)	7.06	6.78		1.04	0.312
Glucocorticoid	0.81	0.89	0.21	0.91	0.370
a. Dependent Variable: Apoptotic cells					
(Constant)	172.49	21.01		8.21	0.000
Glucocorticoid	-6.46	2.74	-0.48	-2.35	0.031
Apoptotic cells	-0.65	0.70	-0.19	-0.92	0.367
a. Dependent Variable: Osteocalcin					

(*) γ : symbol for the path from independent to dependent variable

The effect of osteoprotegerin levels on c-telopeptide levels

The results of the regression analysis showed that there was an effect of changes in Osteoprotegerin levels on C-telopeptide ($p < 0.05$). (Table 4) The results of the analysis

showed a negative path coefficient, meaning that it showed the opposite change, if Osteoprotegerin levels increased then C-telopeptide levels would decrease ($\beta = -0.658$).

Table 4. Coefficient value of osteoprotegerin regression levels to c-telopeptide

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error			
(Constant)	7.04	1.03		6.80	0.000
Osteoprotegerin	-0.04	0.01	-0.65	-3.70	0.002
Dependent Variable: C-Telopeptide					

(*) β : symbol for the path from dependent to dependent path

The effect of glucocorticoid and apoptotic osteoblasts on osteocalcin

The results of the regression analysis showed that there were effects of glucocorticoid levels on Osteocalcin levels ($p < 0.05$). If there is an increase in glucocorticoid levels, it will reduce the levels of Osteocalcin ($\beta = -0.486$). Regression analysis showed that there was no significant effect on changes in the number of Apoptotic Osteoblasts on Osteocalcin levels ($p > 0.05$). The results of the analysis show the value of the negative path coefficient, meaning it shows the opposite change. If there is an increase in the number of Apoptotic Osteoblasts, it will reduce the levels of Osteocalcin ($\beta = -0.191$). (Table 3)

The effect of c-telopeptide and osteocalcin levels on the c-telopeptide / osteocalcin ratio

The results of the regression analysis showed that there was an effect of changes in c-telopeptide levels to the c-telopeptide/osteocalcin ratio ($p < 0.05$) and osteocalcin levels to the c-telopeptide/osteocalcin ratio ($p < 0.05$). (Table 5) The results of the path analysis show a positive path coefficient, meaning that if there is an increase in c-telopeptide levels, it will increase the ratio of c-telopeptide/osteocalcin ($\beta = 0.825$). As for the osteocalcin levels to the c-telopeptide/osteocalcin ratio, the analysis shows a negative path coefficient, meaning that it shows the opposite change if the osteocalcin levels decrease, the c-telopeptide / osteocalcin ratio will increase ($\beta = -0.225$).

Table 5. Coefficient value of C-telopeptide regression and osteocalcin levels to the ratio of C-telopeptide and osteocalcin

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error			
(Constant)	0.01	0.00		2.03	0.058
C-Telopeptide	0.01	0.00	0.82	10.81	0.000
Osteocalcin	0.00	0.00	-0.22	-2.94	0.009
Dependent Variable: Ratio of CT/OC					

(*) β : symbol for the path from dependent to dependent path

The correlation between Causal and variables

The pathway that plays a role in increasing the c-telopeptide / osteocalcin ratio was: Physical exercise has an effect on glucocorticoid levels ($p < 0.05$) with a path coefficient value of 0.793. Glucocorticoid levels affect osteoprotegerin levels ($p < 0.05$) with a path coefficient of -0.668. Osteoprotegerin levels affect the level of c-

telopeptide ($p < 0.05$) with a path coefficient of -0.658. C-telopeptide levels affect the c-telopeptide or osteocalcin ratio with a path coefficient of 0.825. Glucocorticoid levels affect osteocalcin levels ($p < 0.05$) with a path coefficient of -0.486. Osteocalcin levels affect the c-telopeptide / osteocalcin ratio with a path coefficient value of 0.225. (Figure 2)

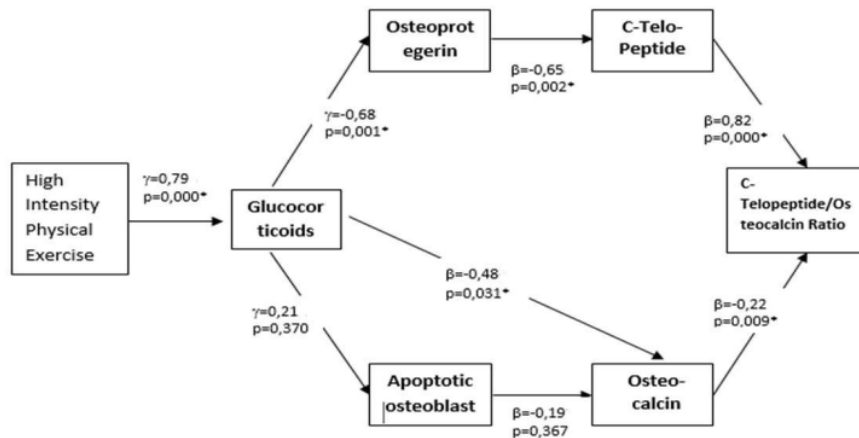


Figure 2. Path Analysis

DISCUSSION

High-Intensity Physical Exercise was proven to affect blood Glucocorticoid levels. This can be seen from path analysis using regression analysis shows a positive path coefficient value of 0.793, meaning exercise High physical intensity increases blood glucocorticoid levels.

In healthy people with high-intensity physical exercise for a long time (12 weeks) will increase the serum concentration of cortisol. Likewise, a survey of 2300 runners competing in the 1987 Los Angeles Marathon showed a 60% increase in the hormone cortisol.[30, 31] High-intensity physical exercise using forced exercise in C3H rats for 9 weeks by treadmill running (15 m/min for 30 minutes/day) increases hormonal responses especially corticosterone compared to sedentary control and treadmill control group (5 m/min for 5 min).[32]

This elevated blood glucocorticoid level is the effect of physical exercise on the Hypothalamus-Pituitary-Adrenal (HPA) Axis. In response to external stimuli that are accepted as a threat of homeostasis (stress), autonomic nervous system activation occurs and plasma cortisol levels tend to rise as a result of activation of the hypothalamo-pituitary-adrenal axis (HPA axis). The hypothalamus hormone corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are the main regulators of corticotropin (adrenocorticotrophic hormone (ACTH) secreted from the anterior pituitary gland. ACTH in turn will stimulate the production and release of cortisol from the adrenal cortical fasciculate zone.[3]

High-intensity physical exercise affects the increased activity of the HPA axis. This happens because the secretion of ACTH and cortisol affects physical exercise more than CRH. In humans, physical exercise is followed by an increase in the release of AVP into circulation

according to the intensity of physical exercise. Increased plasma lactate is one of the causes of HPA axis activation during physical exercise. Other humoral mediators such as angiotensin II and interleukin which increase throughout physical exercise are also able to activate the HPA axis but the role in humans in the activation of the HPA axis is unclear. The activation of the HPA axis was initially described as occurring when aerobic physical exercise exceeds 60% maximum aerobic strength. But in some studies the response of plasma cortisol to physical exercise depends on the pre-exercise baseline conditions.[3]

Other studies have shown that prolonged submaximal of physical exercise and very short high-intensity exercise will activate the HPA axis. High-intensity physical exercise in a short time will increase plasma ACTH levels higher than a submaximal exercise in a long time. The duration of physical exercise also plays an important role in increasing plasma cortisol.[3] This is in accordance with the results of this study, an increase in glucocorticoid levels due to the treatment of high-intensity physical exercise performed in a short time in seconds.

The type of physical exercise also determines plasma cortisol levels. Isometric exercise (resistance) affects the activation of the HPA axis which depends on the intensity of the exercise. The duration and number of reps per set and the length of rest between sets largely determine the cortisol response. Anaerobic physical exercise increases the increase in plasma cortisol rather than aerobic exercise.[3] In general, the response to intensive training in the short term increases plasma cortisol levels, especially anaerobic.[3]

The activity of the HPA axis at the level of the pituitary and hypothalamus have inhibited by cortisol. Sensitivity

to feedback mechanisms is an essential way in the regulation of the HPA axis. The nature and duration of stress can change the sensitivity of cortisol feedback in humans. For example, heavy physical exercise can change the sensitivity of glucocorticoids in peripheral lymphocytes. There are differences in the response of the HPA axis to physical exercise among individuals associated with differences in glucocorticoid sensitivity.[3] After prolonged endurance exercise, normalization of plasma cortisol levels returns after 18-24 hours. This is in accordance with the treatment in the study where physical exercise is done with a frequency of 3 times per week with a daybreak.

Stress causes a higher increase in plasma glucocorticoids above the normal circadian secretion pattern. The height and pattern of plasma glucocorticoid response depends on the nature of the stressor. After finishing treatment, the glucocorticoid concentration immediately returns to the baseline which concludes the existence of a normal circadian.[33] Likewise in this study physical exercise which is a form of physical stressor is to use swimming exercises that cause an increase in blood glucocorticoid levels. The results all animals showed a significant increase in corticosterone (glucocorticoids in rats) in response to high-intensity swimming. High-intensity swimming is a powerful stressor, a combination of physical and psychological aspects.

Corticosterone is secreted by the adrenal glands pulsatile. The exact mechanism (and location of the pulse generator) that underlies pulsatile release is unknown. Possible involvement of PVN (corticotropin-releasing factor pulsatile secretion), sympathetic nervous system and feedback mechanism. The average pulsatile frequency in the hippocampus is once an hour, which is related to the pulsatile frequency of corticosterone in the blood circulation and adrenal glands.[3, 33] In this study described a significant increase in corticosterone after high-intensity swimming compared to placement in a new location as a mild stressor in the control group.

Activation of the sympathetic nervous system and HPA axis due to physical exercise was causes physiological changes that are described as responses to stress. Many libraries state that regulation of activation of acute stress responses facilitates various aspects of fight-or-flight responses. Stimulation of the sympathetic nervous system and HPA axis, for example in increased blood pressure, energy mobilization, and an increase in some immune responses. Many factors affect the stress response of physical exercise including age, gender, health status, living conditions. Types of physical exercise that affect the stress response include intensity, frequency, and duration. The new environment for animal experiments also triggers the stress response, therefore it is necessary to control the environment and these new habits. Control of these new habits can be maximized by choosing the time, speed and duration of training.[34] In this study, rats was received acclimatization for 1 week to adapt to the new environment.

Blood osteoprotegerin levels were lower in rats that received high-intensity physical exercise with significant p. These results are consistent with studies that report that female athletes when compared with sedentary controls, there are increased levels of bone resorption (serum CTX) and decreased serum OPG. In this study the reduction in OPG was due to increased levels of glucocorticoids.[35] Administration of glucocorticoids increases osteoclastogenesis by inhibiting OPG so that the

process of increased resorption causes severe osteoporosis.[15, 23] Whereas other studies have shown women with major depressive disorders. increase bone resorption (serum CTX) and suppress OPG concentrations.[36] Glucocorticoids suppress OPG mRNA expression, especially in human osteoblasts and osteoblast cell lines.[37] Dexamethasone and prednisolone also inhibit OPG production and increase the production of RANKL osteoblasts.[15, 19, 35, 37]

Bone remodeling is strongly associated with osteoblast activity, osteoclasts, cytokines activator of nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG). RANKL bonding to RANK stimulates the formation, differentiation, and activation of osteoclasts and bone resorption. The function of OPG as a decoy receptor that can bind to RANKL plays a role in regulating bone resorption through inhibiting RANKL-RANK interactions. OPG has been shown to be important in the pathogenesis of bone loss in postmenopausal women. Estrogen and physical exercise both affect bone metabolism. In vivo, the absence of estrogen increases the RANKL/OPG ratio, supports osteoclastogenesis and is in harmony with bone loss. Serum RANKL levels increased 20 times in mice without OPG.[38]

There was an increase in the mean apoptotic osteoblasts in the treatment group compared to the control group but statistically did not show significant results ($p>0.05$). (Table 2) There is an effect of high-intensity physical exercise in changes the osteoblast apoptosis. The insignificant results are caused by not checking at the beginning of the study so that the variation in data width or number of samples is too small. Fluid shear stress (FSS) occurs due to mechanical stimulation of bone, activates the PI3-kinase signal, affects the phosphorylation of Akt, and inhibits TNF-alpha. Which induces caspase 3 activation in the execution phase of the apoptotic process, so that osteoblast cells remain alive. [39]

Glucocorticoids will reduce osteoblast precursors or in other words reduce the process of osteoblastogenesis by increasing osteoblast apoptosis.[18] Glucocorticoid administration in mice causes a decrease in bone formation, increases bone resorption and increases osteoclast and osteoblast apoptosis. The existence of a pro-apoptotic effect which is a direct action of steroids on osteoblastic lineage cells, which has been demonstrated through previous research on osteocyte and osteoblast cultures.[18, 40, 41]

This study showed rats that received high-intensity physical exercise had lower osteocalcin levels than control group mice with significant p. In this study, osteocalcin levels decreased with increasing glucocorticoid levels. This can be seen from the path analysis there is the influence of glucocorticoids on osteocalcin which shows the negative pathway coefficient value -0.486 means that with increased blood glucocorticoids will reduce blood osteocalcin levels.

Osteocalcin is one of the non-collagen matrix proteins which is responsive to 1,25-(OH)₂D₃ and is produced mostly by osteoblasts. TNF decreases the expression of osteocalcin mRNA in osteoblast primary culture and osteoblast line cell cloning, which is a transcription repression activity by Nf- κ B. It was also mentioned that IL-1 inhibits the formation and secretion of osteocalcin which stimulates vitamin D through trabecular osteoblasts but not osteoblast periosteum.[10, 42]

High-intensity physical exercise has been shown to significantly reduce blood c-telopeptide levels by p. The

results of the analysis show that high-intensity physical exercise will increase glucocorticoid levels which reduce osteoprotegerin (OPG) levels. Analysis of the OPG pathway to C-telopeptide shows a negative result of -0.658, which means a decrease in OPG levels increases blood c-telopeptide levels.

During the process of bone resorption, osteoclasts release collagen crosslinks resulting from degradation of protein matrix (type 1 collagen) such as pyridinium crosslink (free pyridinoline and deoxypyridinoline), cross-linked telopeptide (N-telopeptides and C-telopeptide) as biochemical markers of resorption or dismantling of bone bone.[8, 13]

A causal correlation that describes the mechanism of imbalance or disruption of bone remodeling where an increase in the c-telopeptide/osteocalcin ratio ($\beta=0.825$) due to an increase in blood c-telopeptide levels directly from a decrease in osteoprotegerin levels due to increased glucocorticoid levels. While osteocalcin levels reduce the c-telopeptide/osteocalcin ratio ($\beta=-0.225$) due to decreased blood osteocalcin levels and increased glucocorticoid levels.

CONCLUSION

The mechanism of bone remodeling on high-intensity physical exercise is caused by the increase of glucocorticoid levels which makes a decrease in osteoprotegerin levels thereby increase c-telopeptide levels.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

SOURCE OF FUNDING

The authors declare that this study was self-funded.

ETHICAL CLEARANCE

This study received a certificate of ethical clearance from ethical commission of Faculty of Veterinary, Universitas Airlangga Indonesia on 7th January 2011, No. 109-KE.

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