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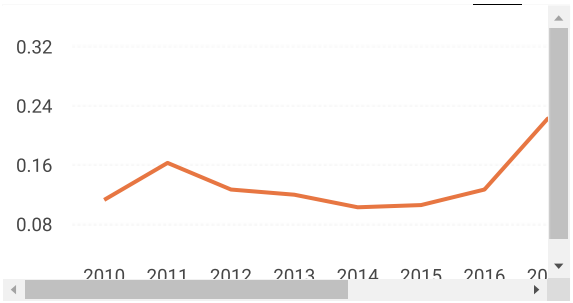

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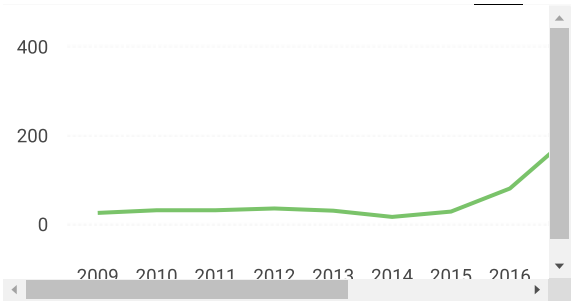

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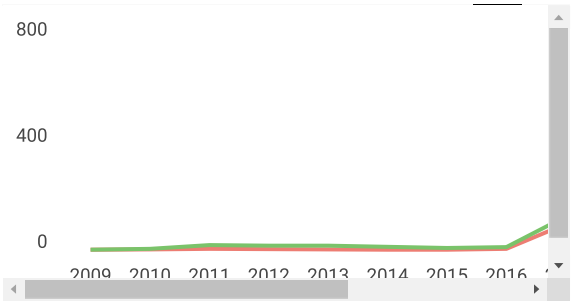

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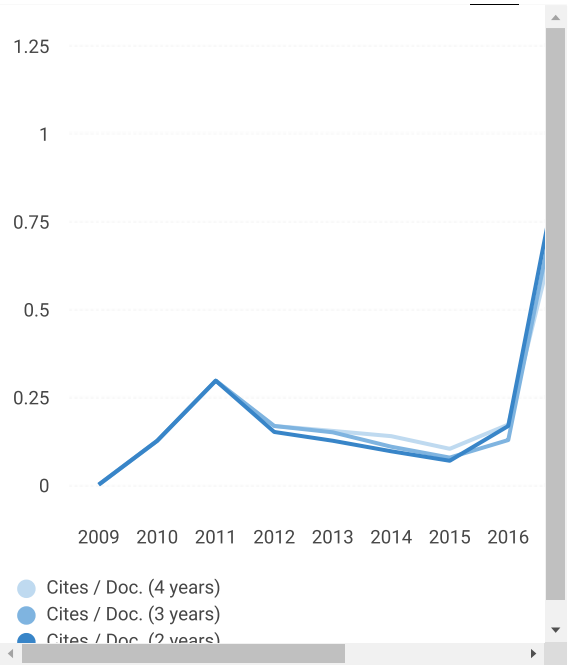

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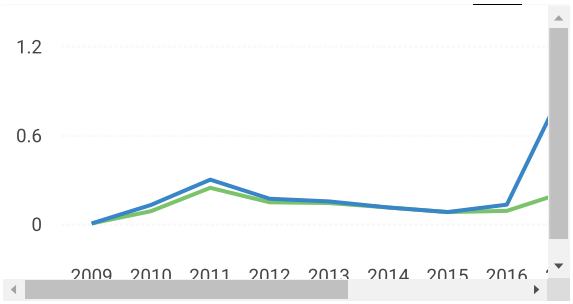

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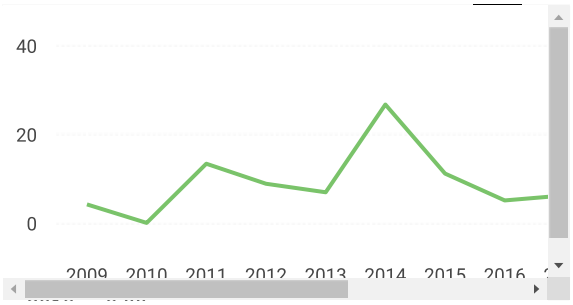

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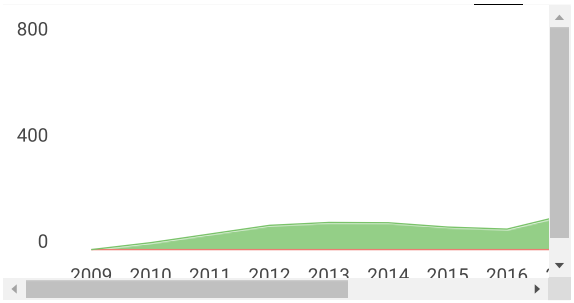

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Acute Effects of Interval and Continuous Physical Activity with Moderate Intensity on Biomarker Bone Formation Procollagen Amino Terminal Propeptide and Bone Carboxy-Terminal Crosslink Telepoptide

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Abstract

Physical activity can affect the bone remodeling process and has an impact on bone health. The bone remodeling through a coupling process consisting of formation and reabsorption. The process stimulates molecules to release collagen into the circulation, i.e., P1NP (osteoblast marker) and CTx (osteoclast marker). However, the acute effect of continous and interval physical activity at moderate intensity on P1NP and CTx has not been recognized yet. This study aims to analyze the disparity of acute effects caused by intervals and continous physical activity with moderate intensity during the growth period of the bone.

This study uses a pre-test and post-test design experimental study. The total sample is 16 which were divided into two groups (interval physical activity group and continous physical activity group).

There was no acute effect on the moderate intensity of continous physical activity on P1NP (p=0.759), but it has shown a decline. Likewise, moderate intensity of acute interval physical activity has shown no effect on P1NP (p=0.013). This phenomenon happened in the moderate intensity of continous physical activity on CTx (p=0.173), as well as the moderate intensity of interval physical activity on CTx (p=0.693). The study revealed no difference in the acute effect of continous and interval physical activity with moderate intensity on P1NP (p=0.209) and CTx (p=0.164).

The acute effect of interval physical activity on moderate-intensity can decrease P1NP levels. Hence, continous physical activity was better than interval physical activity.

Experimental article (J Int Dent Med Res 2023; 16(1): 124-130)

Keywords: Bone remodelling, CTx, P1NP, interval and continuous physical activity, moderate-intensity, medicine.

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Introduction

Data from the Adjust platform shows that the number of health and fitness app installs increased by 67% in March 2020, followed by a 48% increase in the number of sessions in May. In 2021 the number of installations will not be as high as in 2020, but the number of sessions is increasing, this is estimated to be a result of the implementation of Lockdown areas in several countries due to Covid-19.[1]

In the use of fitness applications, the thing that needs to be considered is the compliance or discipline of the user, because it still cannot be monitored. The user's obedience or discipline in running the program will greatly affect the health and condition of the body which is the main purpose of installing the application. Physically, exercise will affect the organs of the body including vital organs, muscles, and bones. Muscle mass is the object most often associated with exercise because of its direct visible effect, but exercise also has a strong influence on bone including the coupling process (formation and reabsorption) of bones that occur simultaneously, however, there are certain conditions that result in an imbalance of the process, which can lead to osteoporosis.[2]

Compared to people in African countries, bone density in European and Asian people are lower, so they are prone to osteoporosis. In Indonesia, of the total people who were examined for bone density, 35% were normal, 36% showed signs of osteopenia and 29% had

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osteoporosis.[3] Thus, prevention methods are needed to decrease the risk of osteoporosis. One method that can be used is by doing regular physical exercise and a calcium diet. Consuming adequate calcium intake and continous weight-bearing exercise are ways to control the risk factor of osteoporosis since it can drive to reach Bone Mineral Density (BMD), both in children and adolescents.[4] Osteoporosis treatment can be done by increasing mass bone, either decreasing bone resorption, increasing bone deposition (formation), or modulating the balance of these strategies.[5,6]

Continuous moderate-intensity treadmill exercise for five weeks in young mice (8 weeks of age) can improve bone cortical, compared to older mice (36 weeks of age).[7] Moreover, treadmill exercise with interval rhythm for ten weeks in eight-week mice rose the BMD and osteocyte lacunar occupancy in subchondral bone.[8] High-Intensity Interval Training (HIIT training) for eight weeks in a woman aged 22 years increased osteopontin.[9]

A study revealed that one plyometric training session in boys and men had stimulated bone formation, demonstrated by an increase in Bone-specific Alkaline Phosphatase (BALP) and Osteoprotegerin (OPG).[10] On the other hand, moderate intensity of continuous physical activity has been claimed as an effective intermediary for circulating the level of bone remodeling, osteocalcin, in obese and overweight conditions.[11]

Meanwhile, acute treadmill exercise with heavy intensity increases bone resorption by increasing CTx (C-terminal telopeptide) and without increasing P1NP (N-amino terminal procollagen propeptide) level, a marker of bone formation.[12]

A P1NP and CTx are bone remodeling markers as they have various advantages over other biomarkers.[13] However, from the multiple studies above, the effect of moderate-intensity interval acute exercise on bone biomarkers is not well known. The comparison of moderate-intensity continuous exercise to bone biomarkers is not clear. This study aimed to determine the differences in the effects of moderate-intensity acute continuous training and moderate-intensity interval training during the growth period.

Materials and methods

This study was conducted by experimental design using the pre-test and post-test approach. This research was conducted at the Faculty of Sports, the State University of Malang, and the University of Brawijaya physiological laboratory, to measure P1NP and CTx and took approximately six months beginning from June 2019. The procedures in this study were carried out by relevant guidelines and regulations. Research data retrieval scheme can be seen in Figure 1. The population was students at the State University of Malang who were suited to the criteria. The researchers made inclusion criteria, including male sex, fulfilling healthy criteria (doctor's examination), age between 21-25 years, willingness to fill out information for consent and to agree to be a research subject, having an average rest HR 60-80 BPM, having a normal BMI 18.5-22, 9 kg/m², not a trained person, being able to perform the movements contained in the test. However, those who have upper or lower limbs and cardiovascular system disease were excluded from the study. The dropout criteria consisted of experiencing injury, dizziness, and nausea while carrying out the test and being unable to attend the examination.

After recruiting the subject, informed consent was obtained from all subjects. As many as sixteen subjects have been involved in this study. Every blood from the subjects was collected before and after the training and tested in the laboratory to determine the P1NP and CTx. The two groups warmed up before starting the implementation phase of the study, namely by doing a treadmill at an intensity of 50% -55% HRMax for 5 minutes. The interval group did treadmill with an intensity of 65% -70% for the first 2 minutes, continued with core activities (Run 6**5%-70% HRmax)** for 4 minutes interspersed with active recovery for 3 minutes at an intensity of 50% -55%, carried out in 4 sets. Whereas, the continuous group did treadmills continuously with core activities at an intensity of 65% -70% for 30 minutes.

One hour after the treadmill activity was completed, a post-test was carried out on both treatment groups, and blood was drawn for each subject to examine Procollagen Type I N-terminal Propeptide (P1NP) and Carboxy Terminal Crosslink Telepoptide (CTx) using the ELISA method. Furthermore, the data that comes out

will be analyzed using the Statistical Package for the Social Sciences (SPSS) v22 for Windows (IBM Inc., Chicago, IL) with considered statistically significant when the value was p<0.05. Descriptive statistical tests carried out the first data processing to describe the overall data obtained in tabular form.

The second data processing is the normality test using Shapiro Wilks (p> 0.05). The homogeneity test used the Levene test (p> 0.05), where if the data were normally distributed, the Paired T-Test was carried out to test the pre and post of each group. Then proceed with the Independent T-Test (T-Test 2 Samples) with a significant level (p <0.05). Meanwhile, the Mann-Whitney test is performed if the data distribution is not normal (p <0.05).

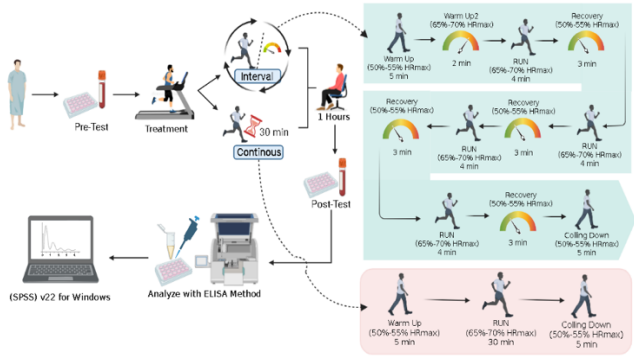

Figure 1. Research Data Retrieval Scheme.

Results

General Characteristics

The characteristics of the research subjects were divided into interval groups and continuous groups had the same variation in characteristics. There were no significant differences in the variables of age, weight, height, and BMI (**Table 1**).

Variable	n	Interval			Continous			p-sig
		Min	Max	Mean ± SD	Min	Max	Mean ± SD	
Age (year)	8	23	24	23.5 ± 0.53	21	25	22.75 ± 1.64	0.055
Weight (Kg)	8	51	63	57.13 ± 3.98	56	70	62.5 ± 4.14	0.925
High (Meter)	8	1.6	1.7	1.66 ± 0.039	1.65	1.78	1.7 ± 0.04	0.240
IMT (Kg/m²)	8	19.03	21.83	20.59± 1.037	19.84	21.58	21.58 ± 0.98	0.855

* Significant if p > 0.05

Table 1. The descriptive statistics of control variable data.

Normality test

After being processed from descriptive statistics, control variable data (height, weight, and BMI) were tested for normality through the Shapiro Wilk test to determine the hypothesis test is carried out parametrically or non-parametric. The results are presented in the form of a bar chart as follows (Table 2 and Figure 4)

Group	n	Test time	P1NP (ng/mL) Mean ± SD	p Normality P1NP	CTx (ng/mL) Mean ± SD	p Normality CTx
Interval	8	*Pre-test*	370.375 ± 89.132	0.942	0.451 ± 0.302	0.924
	8	*Posttest*	288.375± 69.496	0.596	0.524 ± 0.284	0.102
Continue	8	*Pretest*	316.656 ± 98.497	0.190	0.930 ± 0.566	0.221
	8	*Posttest*	302.5 ± 60.371	0.053	0.559 ± 0.398	0.117

Table 2. Shapiro Wilk P1NP & CTx test results.

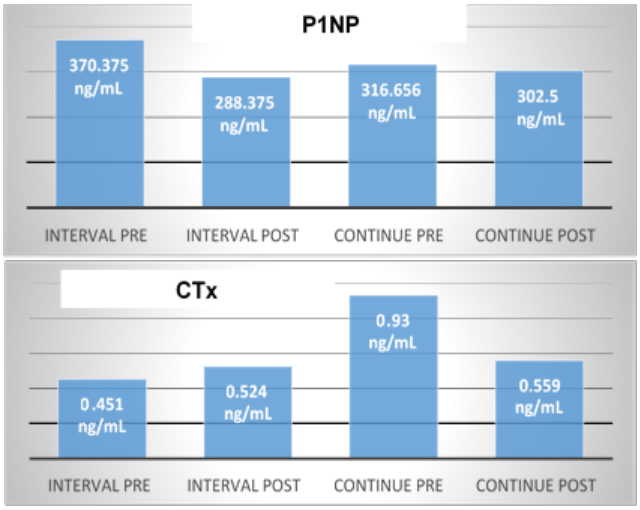

Figure 2. the result of P1NP and CTx serum.

Data for the P1NP and Ctx variables from the interval and continuous groups are typically distributed (p> 0.05) so that they are tested parametrically through the independent t-test.

Paired Sample T-test

The paired sample T-test was conducted to determine the differences before and after the physical activity protocol (interval, and continuous training) by comparing the results of the pre-test and post-test of the two groups.

Group	n	Test time	P1NP (ng/mL) Mean ± SD	p Paired T-Test P1NP	CTx (ng/mL) Mean ± SD	p Paired T-Test CTx
Interval	8	Pre-test	370.375 ± 89.132	0.013	0.451 ± 0.302	0.693
	8	Post-test	288.375 ± 69.496		0.524 ± 0.284	
Continue	8	Pre-test	315.656 ± 98.497	0.759	0.930 ± 0.566	0.173
	8	Post-test	302.5 ± 60.371		0.559 ± 0.398	

* Significant if p < 0.05

Table 3. The Paired Sample T-Test P1NP and CTx T-Test.

Based on Table 3, In the interval group, the mean pre-test results were higher than the post-test results carried out 1 hour after treatment (370,375> 288,375 ng / mL); these results also showed that there was a significant difference between pre-test and post-test (p <0.05). Whereas for the continuous group, the mean pre-test results were also higher than the post-test results 1 hour after treatment (315,656> 302.5 ng / mL), but there was no significant difference between the pre-test and post-test results (p> 0.05). This indicates that continuous treadmill physical activity for up to 1 hour of rest affects the P1NP level in serum.

Whereas in the CTx test, the mean pre-test for the interval group was lower than the post-test they have carried out 1 hour after treatment (0.451 <0.524 ng / mL), but there was no significant difference between the pre-test and post-test (p> 0.05). Whereas for the continuous group, the mean pre-test results were higher than the post-test (0.930> 0.559 ng / mL), but there was no significant difference between the pre-test and post-test (p> 0.05). This indicates that up to 1 hour of rest treadmill intervals of physical activity does not affect serum CTx levels. The result of P1NP and CTx serum shown in Figure 2.

Independent T-test

Different tests were conducted to examine the differences in the effect of the activity intervention interval method treadmill with continuous method treadmill activity on the P1NP and CTx variables (Table 4).

Group	n	ΔP1NP Mean ± SD	*P* Normality ΔP1NP	*P* Independent t-test of ΔP1NP	ΔCTx Mean ± SD	*P* Normality ΔCTx	*P* Independent t-test of ΔCTx
Interval	8	-82 ± 70.304	0.292	0.209	0.073 ± 0.506	0.657	0.164
Continous	8	-14.156± 125.301	0.402		-0.371 ± 0.691	0.501	

* Significant if p < 0.05

Table 4. Independent T-Test in ΔP1NP and ΔCTx.

The difference between the P1NP pre-test and post-test both in the interval and continuous groups showed that the data were normally distributed (p> 0.05). Furthermore, the mean ΔP1NP value in the interval group was lower than the continuous group (-82 <-14.152 ng / mL). Hence, there was no significant difference between the interval and continuous groups (p> 0.05). On the other hand, the difference between pre-test and post-test CTx between the interval and continuous groups showed normal distribution (p> 0.05). The mean of ΔCTx value in the interval group was higher than the continuous group (0.073>-0.371 ng/mL). However, there was no significant difference between the interval and continuous groups (p> 0.05). Based on the different tests between groups above, there is a tendency that continuous physical activity with moderate intensity is better than the moderate intensity of interval physical activity.

Discussion

The T-test showed a significant difference in the interval group before and after treatment (p=0.013 <0.05), which indicated the interval method treadmill physical activity up to 1 hour of rest affected P1NP levels in serum. The results are almost the same as the research conducted in 2017, which stated ultra-marathon running activities in the mountains reduced P1NP levels by 15%.[14] Acutely, the decrease in bone formation markers can be caused by the rapid metabolic response of the bone and the increased energy demands on the muscles. The study stated that there was an increase in CTx and a decrease in P1NP due to low energy availability, which was caused by moderate-intensity running physical activity in active women.[15]

The bone remodeling requires significant energy for the skeleton to grow and maintain strength. Bone activity is carried out by osteocytes, osteoblasts and osteoclasts, which allow the hard tissue to combine its flexibility and capacity to support sustained mechanical stress by releasing calcium into the circulation. This process requires a significant substrate to produce ATP, which is used for osteoclasts to resorb bone and osteoblasts to synthesize collagen.[16] The decrease in bone formation markers is the presence of an inflammatory phase resulting from physical activity. Increases in IL-1, TNF-α and IL-6 are associated with increased osteoclast differentiation by promoting RANKL and OPG production.[17] Hence, TNF-α inhibits the stimulation of preosteoblasts so that the bone formation process decreases, and simultaneously of IL-6 stimulates preosteoclasts and increases bone resorption.[18]

The T test in this study, there was no significant difference in the interval group before

and after treatment (p = 0.693 > 0.05). This indicates that interval treadmill physical activity up to 1 hour of rest has no effect on CTx levels in serum. However, based on the mean value, it showed a different trend in the pretest value of 0.451 ng/ml and for the post-test it showed a value of 0.524 ng/ml. This is in line with other studies which prove that physical activity running high-intensity intervals increases CTx levels 1 hour after treatment. Still, there is a sharp increase at 5 minutes after treatment. In this study, it was explained that new CTx levels decreased after resting for 24 hours. Acute physical activity on the ergometer cycle for 60 minutes showed an increase in CTx levels after recovery for 30 minutes and a peak after 60 minutes of recovery.[19] This case is related to the intensity, duration and type of exercise performed. Intensive exercise with moderate intensity can increase CTx levels.[20] Mezil et al, revealed that bone resorption markers such as CTx showed an increase in bone resorption immediately after low impact physical activity. Because this study used moderate intensity physical activity and measurements were made after 1 hour of treatment, there was still an increase in CTx levels.[18]

In this study, there was no significant difference in the continuous group before and after treatment indicated that the continuous method of treadmill physical activity up to 1 hour of rest did not affect P1NP levels in serum. The results of this study are in line with the research conducted by Scott et al, running training with an intensity of 65-70% VO max did not affect P1NP for up to 2 hours of recovery. The protocol for physical activity running on a treadmill did not affect the increase of bone formation biomarkers up to the next 96 hours.[21] The effect of physical activity on bone biomarkers can be shown through body impact rather than exercise intensity.[22] One plyometric training session in children and adult men can stimulate biomarkers of bone formation.[10] Eccentric exercises are known to increase markers of inflammation and cause muscle damage, and the response of bone biomarkers may be caused by increased inflammation and muscle damage.[22]

A non-significant difference was also found in CTx level serum the continuous group before and after treatment (p=0.17>0.05). When doing continuous treadmill up to 1 hour of rest, CTx serum levels fall low. The results in this study supported by Bemben et al, that CTx did not experience a significant decrease after resistance training, but a difference in trend.[23] The mean difference is probably due to the continuous physical activity performed. Continuous physical activity will cause physical stress on the body and will stimulate the hypothalamus to activate the HPA axis (Hypothalamic Pituitary Adrenal axis) and the sympathetic nervous system. After the HPA axis is active, there is an increase in ACTH secretion, which stimulates cortisol in the adrenal cortex.[24] Activation of the HPA axis can lead to suppression of GH/IGF-1.[25] However, an acute increase in plasma GH concentration can occur at the onset of a stress response or after acute administration of glucorticoids. GH through IGF-1 stimulates the proliferation of epiphyseal cartilage to form more space for bone formation and stimulates osteoblast activity.[26]

Osteoblasts and their immature precursors produce two chemical signals that regulate osteoclast development and activity in opposite ways - the RANK ligand (Reactor Activator Nuclear Kappa-β) and osteoprotegerin. The RANK ligand binds to RANK will trigger osteoclast activity; on the other hand, osteoblasts also secrete osteoprotegerin (OPG) to attach to the RANK ligand and inhibit the RANK ligand from binding to RANK, so that this activity will prevent osteoblast cell apoptosis and suppress osteoclast activity.[27] Because of this, the CTx showed a decreased mean trend from pre-test to post-test due to the continuous physical activity on the treadmill performed.

Based on the independent sample T test in this study, there was no significant difference between the interval group and the continuous group on P1NP levels (p = 0.209 < 0.05). This indicates that the acute effect of continuous exercise and moderate intensity intervals with 1 hour of rest does not have a significant difference on P1NP levels in serum. However, based on the mean value, there was a difference in the interval group value to -82 ng/ml, and for the continuous group, it showed a value of -14.156 ng/ml. Based on this value, it can be said that continuous or interval acute physical activity cannot increase the P1NP level after 1 hour of rest. However, interval physical activity has a more significant decrease than continuous physical activity because there is a difference in the mean value.

Based on these data, both interval and

continuous physical activity of moderate intensity cannot increase the bone formation process. The effect of physical activity on bone biomarkers can be demonstrated through body impact rather than exercise intensity.[22] To increase bone formation (in this case, P1NP), physical activity is needed. Aerobic exercises, such as walking and running, produce less stress on the body, although fatigue on the bones.[8] Thus in this study, neither the moderate-intensity interval nor continuous physical activity using a treadmill could not stimulate bone formation, as measured by P1NP.

Based on this value of the independent sample T-test, continuous acute physical activity can reduce CTx levels than interval acute physical activity because there is a difference in the mean value, even though it does not show a significant difference. Similar research revealed that 1.5 hours after physical activity drop jumps could reduce CTx levels in active men.[28]

In another study, jogging activity for 30 minutes at an intensity of 60-70% heart rate can reduce the levels of bone resorption biomarkers 1 hour after treatment, which was carried out in young women.[29] The decrease in CTx could be due to increased OPG secretion after carrying out the physical activity protocol. Physical activity drop jumps in boys and men increases OPG secretion 1 hour after treatment. In this case, the rise in OPG will prevent osteoblast apoptosis and decrease osteoclast activity; hence bone resorption also decreases.[10]

The difference in bone response to exercise depends on the type of exercise, intensity and used protocol. The kind of physical activity undertaken, the right frequency and duration will increase the bone formation and inhibit the bone resorption, thereby increasing bone density.[30] On the contrary, running on a treadmill with heavy and tiring intensity increases levels of bone resorption, CTx.[21]

This is because physical activity can increase the formation of free radicals, increasing oxidative stress.[31] Oxidative stress activates differentiation of preosteoclasts in osteoclasts and strengthens bone resorption during inflammation.[32] The above studies are inversely related to this study, which tends to decrease CTx from pre-test to post-test.

Conclusions

The acute effects of continuous and Interval physical activity with moderate intensity cannot increase P1NP levels. The acute effect of moderate-intensity interval and continuous physical activity cannot reduce CTx levels, but in continuous group there is a difference in the trend of the mean CTx before and 1 hour after physical activity. There was no difference between the acute effects of moderate intensity interval and continuous physical activity on P1NP and CTx levels. However, there is a trend that shows that continuous physical activity is better than interval physical activity with both moderate intensity

Declaration of Interest

The authors report no conflict of interest.

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