

The effect of measurable and regular exercise on ovariectomized Sprague Dawley rats in improving skin quality

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ABSTRACT

The decline of estrogen level causes various skin changes including amount of fibroblast, the thickness of epidermis and dermis. Physical exercise is believed can increase the estrogen level and give benefit impacts on skin. Its mechanism is often associated with the increase of extragonadal aromatization and estrogen serum, the activation of insulin-like growth factor (IGF), and the expression of estrogen receptor (ER). This study was conducted to investigate the effect of physical exercise in ovariectomized rat on the amount of fibroblast, the thickness of epidermis and dermis, the levels of ER β in skin and serum estrogen. Eight female Sprague Dawley rats aged 3 months were used in this study. Rats were divided into two groups i.e. Group 1 consisted of ovariectomized rats without physical exercise and Group 2 consisted of ovariectomized rats and given measurable and regular physical exercise. Rats ran on treadmill with the speed 18 m/min, the tilt of 5%, duration for 60 min/experiment/day, 5 times a week for 12 weeks. The amount of fibroblast, the thickness of epidermis and dermis, the levels of ER β in skin and serum estrogen were measured after physical exercise. The result showed that there was a significant difference amount of fibroblast between group 1 and group 2 ($p < 0.05$). However, no significant difference the levels of serum estrogen, ER β , and the thickness of epidermis and dermis between Group 1 and Group 2 ($p > 0.05$). There was a significant positive correlation between the level of serum estrogen and the thickness of epidermis ($p < 0.05$), and negative correlation between the serum estrogen level and the level of ER β ($p < 0.05$), and negative significant correlation between the level of ER β and the thickness of epidermis ($p < 0.05$). In conclusion, the amount of dermal fibroblast of ovariectomized rats increase after underwent measurable and regular exercise. There is correlation between the serum estrogen level and the thickness of epidermis as well as ER β .

ABSTRAK

Penurunan kadar estrogen dapat menyebabkan perubahan kulit termasuk jumlah fibroblast, ketebalan epidermis dan dermis. Latihan fisik dapat meningkatkan kadar estrogen dan berdampak positif pada kulit. Mekanismenya berhubungan dengan peningkatan aromatisasi ekstragonadal, peningkatan estrogen serum, aktivasi *insulin-like growth factor* (IGF), serta ekspresi reseptor estrogen (RE). Penelitian ini bertujuan mengkaji pengaruh latihan fisik pada tikus yang dilakukan ovariektomi terhadap jumlah fibroblast, ketebalan epidermis dan dermis, kadar RE β kulit, dan kadar estrogen serum.

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Delapan ekor tikus Sprague Dawley betina umur 3 bulan digunakan dalam penelitian ini. Tikus dibagi menjadi dua kelompok yaitu Kelompok 1 tikus dilakukan ovariectomi dan tidak diberi latihan fisik dan Kelompok 2 tikus dilakukan ovariectomi dan diberi latihan fisik teratur dan terukur. Tikus berlari di atas *treadmill* dengan kecepatan 18 m/menit, kemiringan 5%, durasi 60 menit/kali/hari, dan frekuensi 5 kali per minggu, selama 12 minggu. Setelah latihan fisik teratur dan terukur, jumlah fibroblast, ketebalan epidermis dan dermis, kadar RE β kulit dan estrogen serum diukur. Hasil penelitian menunjukkan bahwa terdapat perbedaan bermakna rerata jumlah fibroblast antara Kelompok 1 dan Kelompok 2 ($p < 0,05$). Namun, tidak terdapat perbedaan bermakna untuk rerata kadar estrogen serum, kadar RE β , ketebalan epidermis dan dermis antara Kelompok 1 dan Kelompok 2 ($p > 0,05$). Terdapat hubungan positif yang bermakna antara kadar estrogen serum dengan ketebalan epidermis ($p < 0,05$) dan hubungan negatif antara kadar estrogen serum dengan kadar RE β kulit serta hubungan negatif yang bermakna antara kadar RE β kulit dengan ketebalan epidermis ($p < 0,05$). Dapat disimpulkan, jumlah fibroblas dermis tikus yang menjalani latihan fisik teratur dan terukur lebih besar dibandingkan tanpa latihan fisik teratur dan terukur pada tikus yang dilakukan ovariectomi. Terdapat hubungan antara kadar estrogen serum dan ketebalan epidermis dan juga RE β .

Keywords: physical exercise - serum estrogen - estrogen β receptor - fibroblast - dermis - epidermis.

INTRODUCTION

The decline of estrogen level in postmenopausal woman shows various impacts such as low content of collagen in skin, increase of the numbers and the depth of wrinkles, skin depletion, dryness in skin and low elasticity.^{1,2} In addition, low estrogen level may cause long wound healing and atrophy.³ In young rats after ovariectomy (OVX), visible skin deteriorations are found such as low number of collagen fibers in skin,⁴ the decrease of subcutaneous tissue's thickness, the increase of breaking strength, tensile strength and Young's skin modulus.⁵ The decrease in the thickness of epidermis, dermis and the percentage of dermis collagen are also found in the young rabbits after OVX.⁶

One of most convenient methods to elevate estrogen level is physical exercise. Regular physical exercise with medium intensity is proven can increase the serum estrogen level on rats after OVX⁷ and on post-menopausal women.⁸ Regular physical exercise can also increase the CYP19 expression in adrenal cortex and

fat tissue.^{9,10} Furthermore, estrogen level in cerebellum¹¹ and hippocampus¹² of rats after OVX increases as the result of measurable and regular physical exercise for 12 weeks

Physical exercise gives also significant impact on the expression of estrogen receptor (ER) in liver, heart and muscles.^{7,13} The expression of particular hormone's receptor can change along with the physiologic need of one tissue on that hormone.¹⁴ Physical exercise also elevates the level of insulin-like growth factor-1 (IGF-1).¹⁵ The IGF-1 is promoting cell growth involved in keratinocytes proliferation¹⁶ and fibroblast so that the decreasing number of IGF-1 contributes to the decrease of skin thickness.¹⁷ It is also able to form cross talk (cross interaction which influences each other) with ER. The IGF-1 activates mitogen activated protein kinase (MAPK) cascade which phosphorylates transcription factor leading to increasing the transcription activity of ER.¹⁸ The ER β plays dominant role in regulating the action of estrogen in skin.¹⁹

This study aimed to investigate the effect of measurable and regular physical

exercise on serum estrogen level and ER β level in skin on ovariectomized Sprague Dawley rats. Their effect on the amount of fibroblast and the thickness of epidermis and dermis of the rats was also evaluated.

MATERIALS AND METHODS

Animal model procedure

This was an experimental study using post test only control group design involving eight female Sprague Dawley rats aged about 3 months. Before experimental, rats were adapted in individual cage for 7 days before OVX. Following after OVX, the rats were adapted again for 7 days for OVX wound healing and hormonal adaptation. On the 15th day, these rats were randomly divided into two groups with four rats for each group. Group 1 consisted of rats underwent OVX without physical exercise, whereas Group 2 consisted of rats underwent OVX with regular and measurable physical exercise.

Gradual adaptation of the physical exercise on the rats of Group 2 was conducted on the 15th to 21st day. Furthermore, the physical exercise was initiated for 5 days a week (Monday, Tuesday, Wednesday, Friday, and Saturday) for 12 weeks with medium intensity and the tilt of 5%. The duration of the physical exercise was 60 min consisting 10 min of warming up with the speed of 12 m/min, 40 min for main exercise with the speed of 18 m/min, and 10 min for cooling down with the speed of 12 m/min.⁷ On the 106th day, the rats were anesthetized using HCl ketamine. Following after the rats lose their consciousness, blood and skin samples were taken. The blood sample was taken through orbitalis sinus for the estrogen serum level measurement. Two sample of skin tissues were taken by using 5 mm-punch biopsy on left leg. One fresh the skin tissue sample was used for the skin ER β level measurement and another one was dipped into formalin 10% to produce paraffin block. Furthermore, HE staining was conducted to examine the

amount of fibroblast and the thickness of dermis and epidermis.

Examination of serum estrogen level, ER β , dermis fibroblast and epidermis thickness

The serum estrogen and skin ER β levels were measured by using ELISA technique. The amount of dermis fibroblast was measured in 10 microscope fields of view at a magnification of 200 x after HE staining. The thickness of epidermis and dermis were measured by using Optilab®Image Raster version 2.1 on 10 different places.

Statistical analysis

Data were presented as mean \pm SEM. The different of serum estrogen level, skin ER β level, dermis fibroblast and epidermis thickness of the both groups were analyzed using paired t test. Moreover, the relationship between the variables were analyzed using Pearson correlation. A p value <0.05 was considered to be significant.

RESULTS

Serum estrogen and ER β levels, amount of fibroblast, thickness of epidermis and dermis

No significantly different in the serum estrogen level, skin ER β level and thickness of epidermis as well as thickness of dermis between the rats underwent OVX with physical exercise (Group 2) and those without physical exercise (Group 1) were observed. However, the amount of fibroblast on Group 2 (15.75 ± 1.70) was significantly higher than that on Group 1 (8.75 ± 0.48) ($p < 0.05$) as presented in TABLE 1. It was indicated that the regular and measurable physical exercise on the rats underwent OVX increased the amount of fibroblast, however, did not influence the serum estrogen level, skin ER β level, thickness of epidermis and thickness of dermis.

TABLE 1. Serum estrogen and ER β levels, amount of fibroblast, thickness of epidermis, and thickness of dermis (mean±SEM) in both groups of rats

Variable	Group	n	Mean ± SEM	p
Serum estrogen level (ng/mL)	1	4	24.23±10.99	0.761
	2	4	28.03±4.59	
ERβ level (pg/mL/50 mg tissue)	1	3	0.028±0.067	0.260
	2	4	0.019±0.004	
Amount of fibroblast	1	4	8.75±0.48	0.007*
	2	4	15.75±1.70	
Thickness of epidermis (μm)	1	4	2.93±0.24	0.199
	2	4	3.35±0.17	
Thickness of dermis (μm)	1	4	63.13±2.52	0.112
	2	4	72.43±4.31	

Note: Group 1 : rats underwent OVX without physical exercise; Group 2: rats underwent OVX with regular and measurable physical exercise; *significant (p<0.05)

Correlation between the serum estrogen, ERβ levels and amount of fibroblast, the thickness of epidermis as well as dermis

Negative correlation between the serum estrogen level and the thickness of epidermis (r = -0.832; p = 0.029), positive correlation between the skin ERβ level and

the thickness of epidermis (r = 0.759; p = 0.040) and negative correlation between the serum estrogen level and skin ERβ level (r = -0.911; p = 0.011) were reported in this study (TABLE 2). It was indicated that the serum estrogen level increases the thickness of epidermis. In contrast, the skin ERβ decreases the thickness of epidermis.

TABLE 2. The Pearson Correlation analysis

	Correlation	r	p
Serum estrogen level	Amount of fibroblast	-0.244	0.861
	Thickness of dermis	0.564	0.582
	Thickness of epidermis	-0.832	0.029*
Skin ERβ level	Amount of fibroblast	-0.074	0.641
	Thickness of dermis	-0.231	0.244
Serum estrogen level	Thickness of epidermis	0.759	0.040*
	Skin ER β level	-0.911	0.011*

* significant (p<0.05)

DISCUSSION

The serum estrogen level of the rats underwent OVX with physical exercise (G2) was higher than those without physical exercise (G1), although it was not significantly different (TABLE 1). The mechanism underlying the effect of physical exercise on serum estrogen level is not quite understood. After menopause or OVX, the serum estrogen is produced and secreted by peripheral steroidogenic active glands through extragonadal aromatization process.^{20,21} Due to the following explanation, physical exercise is assumed to increase extragonadal aromatization process. Its mechanism has correlation with the production of IL-6 during performing endurance exercise.^{22,23}

IL-6 is involved in immune response and acute phase reaction. Cytokine that is produced during inflammation can influence the communication between the hypothalamus-pituitary-adrenocortex (HPA) axis and immune system.²⁴ Inflammatory stimuli activates anti-inflammation signals from the central nervous system, whereas inflammation in peripheral tissues triggers neural signals in hypothalamus.²⁵ Acute phase response stimulates the expression of mRNA IL-6R in hypothalamic nucleus causing IL-6 activates HPA axis.²⁶ IL-6 is presumably produced as acute phase response after physical exercise.²⁷ These signal induces hypothalamic releasing factors which eventually enhance the secretion of hormone pituitary including adrenocorticotrophic hormone (ACTH).²⁸

ACTH which is released will result in rapid increase in the formation of pregnenolon and its derivatives, including cortisol and androgen adrenal. In longer period, ACTH elevates the synthesis of P450 which influences the formation of adrenocortex hormones.²⁹ P450 aromatase is an enzyme responsible for converting testosterone into estradiol and androstenedion into estron.³⁰ P450 aromatase can be activated by inflammatory mediator,³¹ one of them is

IL-6,²⁴ so that it increases the capability of extragonadal tissue in synthesizing estrogen from androgen.³¹ Estrogen tissues will join the circulation if they do not take part in local metabolism.³²

The test result on mean of ER β level of group given OVX without physical exercise (G1) and group given OVX and physical exercise (G2) shows that mean of those groups have no significant difference $p > 0.05$ (TABLE 2). However, mean of ER β level of G1 is higher than mean of ER β of G2 (TABLE 2). It means that physical exercise decreases ER β level of skin in rats after OVX. Factors that influence this to happen are not quite understood, but it is assumed that physical exercise influences steroidogenesis process in skin. DHEA is proven to be converted into estradiol in skin.³³ Physical exercise is proven to influence steroidogenesis process in several tissues which eventually elevates local estrogen level in tissues.^{11,12} The increase of estrogen level in local tissues is assumed to contribute to ER β transcription process leading to decreased level of ER β level in skin. Mechanism refers to adaptation of receptor's need on its physiologist need because the adaptation of the number of receptor is very specific in each network.¹⁴

Besides, it is possible that estrogen mechanism in skin after OVX and physical exercise does not frequently involve genomic response. This assumption is who found that estradiol stimulates the production of skin procollagen in rats and human, although it does not activate ER. Thus, it needs further research focusing on estrogen action mediator.³⁴ Mean of the number of fibroblast on group given OVX without physical exercise (K1) compared to group given OVX and physical exercise is significantly different ($p < 0.05$) (TABLE1). Mean of the number of dermal fibroblast of Group 2 is significantly higher than Group 1 (TABLE1). It shows that physical exercise increases the amount of fibroblast in skin. However, the result is still lower than mean of group which gets neither OVX nor

physical exercise.

No research has ever been conducted on examining the influence of physical exercise on the amount of skin fibroblast. However, the mechanism that might happen correlates with the effect of physical exercise on local steroidogenesis process in skin tissue. Local estrogen plays important role on skin physiology especially fibroblast.³⁵ Fibroblast migration and proliferation increase along with the existence of DHEA; however, the effect is hampered by aromatase inhibitor. It shows that fibroblast migration and proliferation depend on the conversion of 17 β - estradiol. Mechanism of intracrine estrogen action in skin fibroblast indicates that skin fibroblast contains an enzyme converting DHEA into estradiol.³³

Fibroblasts and dermal papilla cells express 5 α -reductase type 2.³⁶ Skin fibroblast express P450 (aromatase).^{37,38} However, this research does not measure the level of estrogen in tissues, enzymes and genes involving in steroidogenesis process in skin; therefore, its underlying mechanism can be clearly explained. Research show that measurable and regular physical exercise increase antioxidant enzyme which serves as the protector against skin deterioration due to free radicals.³⁹ The increased expression of catalase enzyme and glutathione peroxidase on rats after 16 week-physical exercise using freewheel running. Glutathione peroxidase and superoxide dismutase on rats increase after swimming.⁴⁰ Therefore, measurable and regular physical exercise elevates the ability to prevent any destruction resulted from free radicals.⁴¹ Physical exercise is also proven to increase the level Insulin-like growth factor-1 (IGF-1).¹⁵ IGF-1 is a growth factor involved in fibroblast proliferation.⁴² Thus, the increased level of IGF-1 elevates fibroblast proliferation.

Physical exercise endurance also significantly increases blood flow.⁴³ The increased microcirculation causing increases tissue oxygenation and nutrient delivery.⁴⁴ This mechanism is assumed to

increase fibroblast proliferation. The result test on mean of the thickness of epidermis and dermis for group given OVX without physical exercise (G1) compared to group given OVX and physical exercise (G2) is not significantly different $p > 0.05$ (TABLE 1 and TABLE 2). It means that measurable and regular physical exercise increases the thickness of epidermis and dermis.

Previous research examining the effect of physical exercise on the thickness of epidermis and dermis has never been conducted before. Mechanism that might happen has correlation with the effect of physical exercise on the local steroidogenesis process in skin tissues. Therefore, local estrogen level will improve the physiology of skin tissue. Moreover, physical exercise elevates antioxidant enzyme will prevent the damage on the component of connective tissue in dermis due to the formation of ROS on skin of OVX-induced rats.^{39,45,46}

The changes of skin mechanical structure is associated with age and period after menopause, as it has been stated.⁵ Therefore, age possibility and the length of time after OVX influence the result of research in skin thickness. The age of rats which get OVX also influences the result.⁴⁶ The uses of Wistar rats aged 5 months for research on the biomechanical decrease on rats skin after OVX.⁵ Thus, the age of rats undergoing OVX also has possibility to affect end result of physical exercise on skin thickness.

Besides, there is a final phase in tissue rejuvenation process, namely the remodeling process. This phase has long duration in which incessant synthesis of collagen happens. Collagen damages are renovated and stabilized in a stable condition for 21 days or even weeks after. Remodeling phase occurs during the first week until the 12th week or more.⁴⁷ Hence, before reaching the last period of remodeling, skin thickening will not stop. When reaching stable extracellular matrix, skin thickness will not lengthen as the time passes by. Based on period after OVX, treatment in this research will stop

in the 12th week because tissue rejuvenation has reached the final phase of remodeling. However, it need time-series based research to ensure this assumption.

A study should be conducted to investigate the relation between ER β level and the thickness of epidermis and dermis as well as serum estrogen level with the thickness of epidermis and dermis. From the correlation test on the relation between ER β level and the thickness of epidermis, the result shows significant correlation. A significant correlation is visible in serum estrogen level with the thickness of epidermis (TABLE 2). The function of epidermis is proven to be regulated by the circulation of estrogen level, even though estrogen metabolism process and estrogen sensitivity in peripheral tissue also greatly contribute to epidermis homeostasis. In epidermis, estrogen plays important role in keratinocyte proliferation.⁴⁸

Receptor upregulation is one of method to scrutinize the response of intracellular receptor on the increased hormone level.⁴⁹ However, this research finds no significant difference on ER β level between G1 and G2. It is possible that estrogen action in skin in order to maintain the thickness of epidermis and dermis genomically occurs through classic pathway depend on ligan. It does not only involve ER β but also ER α ; however, this research will not further discuss this area. Besides, it can also occur through gnomonic pathway which does not depend on ligan; however, the capability of GF to activate ER β is lower than ER α .⁵⁰

CONCLUSION

Amount of dermal fibroblast on rats increases after measurable and regular exercise. The increase on the level of serum estrogen elevates the thickness of epidermis and decrease the level of ER β . The increase on the level of ER β decrease the thickness of epidermis. It is suggested to conduct further study about the influence of OVX and

physical exercise on the expression of skin steroidogenesis enzymes, skin estrogen level and IGF-1 level. The study can be conducted on older rats using much more samples. It can also use time series research, in order to examine the changes on skin structure due to longer period of physical exercise and after OVX.

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