The comparison of the effect of acute moderate and high-intensity exercise on the uncoupling protein -1 secretion

by Desiana Merawati

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Abstract

Physical activities accelerate the secretion of the uncoupling protein-1 (UCP-1), which reduces the risk of metabolic disorders and the prevalence of obesity. This research aimed to investigate the effects of acute physical activities on UCP-1 secretion. A total of 20 males aged between 19-20 years old) were divided into groups with either a 30-minute moderate-intensity exercise (MIE) or high-intensity exercise (HIE). The UCP-1 expression was measured using Enzyme-Linked Immunosorbent Assay (ELISA) (pre and 5 minutes post the exercise). The pre and post-UCP-1 data in each group were compared with a paired t-test, while pre and post UCP data between groups were analyzed using the Independent-Sample T-test. The pre and post test UCP-1 in the MIE was (4.16±0.89) ng/mL and (4.55±1.36) ng/mL ($P>0.05$), while the pre and post test UCP-1 in the HIE were (4.09±0.53) ng/mL and (5.06±1.02) ($P<0.05$). No significant difference in pretest UCP-1, however was found between groups ($P>0.05$) and posted UCP-1 ($P>0.05$). In conclusion, 30 minutes of high-intensity exercise is required to increase UCP-1 in young adults significantly.

Keywords: moderate-intensity, high-intensity, execise, UCP-1

INTRODUCTION

Currently, obesity is perceived as a world pandemic that has affected 600 million adults worldwide (Rodrigues *et al.*, 2018). WHO survey estimates that 10.8 % of men and 14.9% of women encounter obesity with a body mass index ≥ 30 (Appari *et al.*, 2018). The fact obesity belongs to 3 out of 5 high-risk diseases (Kim *et al.*, 2018). The expected obesity prevalence in the Asia Pacific is 11.9%-37.1%, foreseen as the trigger of accelerating cardiovascular diseases (CVD) and type 2 diabetes mellitus prevalence (Herningtyas and Ng, 2019), as well as the prevalence of high blood pressure, dyslipidemia, and at risk of complication (Kim *et al.*, 2018). Besides, it possibly escalates morbidity and premature death (Flouris *et al.*, 2017), and threatens people's economic and social health (Appari *et al.*, 2018). Physical activation increases aerobic metabolism and browning activity (Purdom *et al.*, 2018) thus, it enables increasing UCP-1 secretion (Kim and Plutzky, 2016). The body's response and adaptation to exercise activities are complex. Therefore, suitable and sufficient physical exercises to improve UCP-1 expression is still required.

Exercise is a healthy and fun activity that includes all systems (Huh, 2018). In addition, exercise can also increase fat oxidation and the ability of skeletal muscles to utilize fat (Jabbour and Iancu, 2017). However, an exercise that is done inappropriately has the potential to trigger metabolic disorders (Brondani *et al*., 2012). Metabolic disorders can result in impaired fat oxidation and reduce protein expression in mitochondrial membranes (Huh, 2018). Yet, the right exercise intensity boosts aerobic metabolism and browning activity (Jabbour and Iancu, 2017). Recent research reports that low-moderate physical activities escalate lipid metabolism, but heavy intensity exercises tend to decrease lipid metabolism (Oh *et al*., 2017). In conclusion, exercise intensity and duration correlate with fat metabolism transforming protein composition in mitochondria and causing an efficient oxidation process by altering H proton going through the membrane to transport electrons used for managing ATP synthesis (Befroy *et al*., 2008). The increase in lipid metabolism is initiated by the acceleration of irisin secretion produced by escalated muscle contraction during physical activities (Brondani *et al*., 2012).

Expansion of irisin drives increasing browning (Oh *et al*., 2017) activating UCP-1 expression so that the thermogenesis is improved (Brondani *et al*., 2012). However, not all physical activities accelerate UCP-1. It is reported that 12 weeks of endurance exercises in healthy and prediabetic adults subject gives no significant effect on fat oxidation (Zhang *et al*., 2017). Besides, physical activities do not affect white adipose tissue (Dinas *et al*., 2017). UCP-1 is located inside the mitochondrial membrane of brownish adipose. It acts as a transmembrane protein that forces ATP synthesis through fat oxidation and thermogenesis that relies on exercise (Fenzl and Kiefer, 2014). Continual moderate and high-intensity physical activities increase UCP-1 expression and gene expression from fatty acid transportation (Khalafi *et al*., 2020a). However, the 60%-64% VO2max intensity exercise on the trained person and 47%-52% VO2max intensity exercise on the untrained person extend fatty oxidation (Oh *et al*., 2017).

In contrast, running with 100% aerobic speed better repairs fatty oxidation and body composition than moderate intensity (Jabbour and Iancu, 2017). Other researchers state that a long duration of moderate exercise is perceived as effective for fatty oxidation (Purdom *et al.*, 2018). Yet, research also reveals that six weeks of moderate-intensity exercise produces no difference in UCP-1 expression activity (Dinas *et al.*, 2017). In other words, fatty oxidation is not only affected by intensity, duration and types of physical activities but also the training, obesity level, types of obesity (visceral or subcutaneous fat), food supply (before or after meal) and the food composition (fat percentage), as well as the genetic factors (Oh *et al.*, 2017). Chronic and acute physical activities activate brown fat (Gorski *et al.*, 2018) by increasing PGC-1a on skeletal muscle (Dinas *et al.*, 2017). Therefore, this study examines the effect of high and moderate physical activities on the expression of UCP-1 in young adults to decrease and prevent the accelerating obesity prevalence. The subjects used in this study have criteria, namely male, aged 19-20 years, have a good level of physical fitness, the subject is a trained person but not an amateur or professional athlete.

METHODS

Study design

This study uses a true experimental method with a randomized pretest-posttest group design. A total of 20 19-20 years old young adults, body mass index (BMI) of 18.5-24.9 kg/m2, normal blood pressure, regular resting heart rate (RHR), excellent physical freshness level, and healthy hemoglobin (Hb) contribute to studies. They were randomly divided into two groups: AMIP (n=10) and AHIP (n=10). The Ethical Committee approved all procedures of the study of the Faculty of Medicine, Brawijaya University, Malang, with registration number 106/EC/KEPK/04/2018.

Physical activity protocol

The exercise was completed by running on a treadmill for 40 minutes (Rejeki et al., 2021; Rejeki et al., 2022; Andarianto et al., 2022) with a high-

intensity (75-85% HRmax) and moderate-intensity (65-75% HRmax). Intensity measurement was carried out using a measurement formulation of HRmax: HRmax – age in years (220 – age in years) (Sugiharto *et al.*, 2021; Tsuchiya *et al.*, 2014). During that activity, the subjects' heartbeat rates were monitored by the polar heart rate monitor.

Data collection

The assessment of participants' anthropometry, physiology and physical fitness involved body height, body weight, body mass index (BMI) and blood pressure, Hb and physical fitness measurement with a multi-stage fitness test (MFT) 20 M (Paradisis *et al.*, 2014; Sugiharto *et al.*, 2022).

In addition, the blood draw for UCP-1 expression analysis from the cubital vein (Daskalopoulou *et al.*, 2014) was performed during the before-exercise dan 5 min after exercise (Huh *et al.*, 2014). After that, the blood was centrifuged. The serum was divided and saved at -80°C temperature (Tsuchiya *et al.*, 2015). The UCP-1 secretion measurement was accomplished using ELISA (Catalog No. E-EL-H1661; Elabscience, Inc., China) (Adji *et al.*, 2021; Sugiharto *et al.*, 2021).

Statistical analysis

Data were analyzed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). The data was first tested using a descriptive test, then the data was tested for normality using the Shapiro Wilk test. Furthermore, in assessing the differences between those moderate and high-intensity exercises between pre or post-test using Paired Samples T-test, with a significance level of ($P<0.05$), meanwhile to assess differences between groups, the Independent-Samples T-test was performed, with a significance level of ($P<0.05$).

Research procedure

The time it takes the issue to provide treatment, i.e. one intervention with high and moderate intensity treadmill exercise for 40 minutes. The technique used for taking the subject is using the Consecutive Sampling technique. The division of groups is done by matching and then using a zig-za system. Data collection is done by checking the level of UCP-1 between

before and 5 minutes after the intervention using the ELISA Kit. The treatment was observed by monitoring heart rate during exercise using a POLAR heart rate monitor H10.

RESULTS

The descriptive analysis of each participant's body profile is presented in Table 1.

Table 1. Characteristics of the Subjects

Variable	n	Group		*p-value*
		MIE (*n*=10)	HIE (*n*=10)	
Age (years)	10	20.40±0.69	20.20±0.79	0.145
Body Height (m)	10	1.65±0.05	1.67±0.04	0.586
Body Weight (kg)	10	60.20±5.18	59.50±6.48	0.570
BMI (kg/m^2)	10	22.06±1.52	21.25±1.40	0.092
SBP (mmHg)	10	118.00±4.22	114.00±8.43	0.254
DBP (mmHg)	10	81.00±3.16	77.00±4.83	0.120
RHR (bpm)	10	72.40±10.06	70.80±8.44	0.911
VO_{2max} (mL/kg/min)	10	42.65±2.72	42.78±3.08	0.992
Hb (g/dL)	10	15.38±1.33	16.40±2.41	0.625

Independent-Sample T-Test. Data are presented as mean±SD. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; RHR: Resting heart rate; VO_2max: Maximum oxygen volume; Hb: Hemoglobin; MIE: moderate-intensity exercise; HIE: high-intensity exercise.

The Independent-Sample T-test presented in Table 1 displays no essential differences among the healthy subjects' characteristics in both groups. Additionally, the results of the UCP-1 secretion analysis between before and 5 min after exercise in each group are shown in Figure 1.

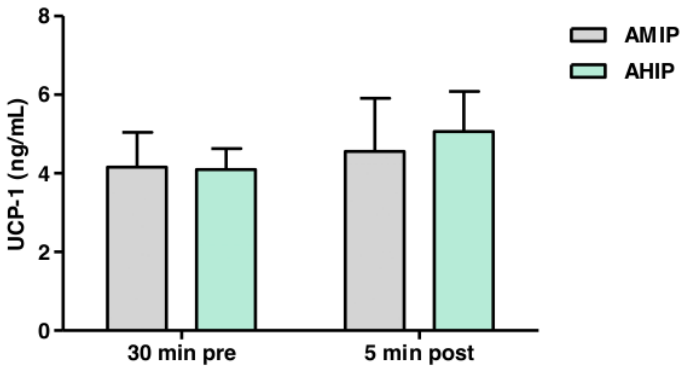

Figure 1. Graphic of average UCP-1 expression analysis between MIE and HIE

Based on the results of the normality test in Figures 1 and 2 show that the data has a normal distribution (P>0.05). The Independent-Sample T-test in Figure 1 exhibits no significantly different average UCP-1 secretion between MIE vs. HIE on pre (P>0.05) and post-test (P>0.05). The results of the analysis of UCP-1 secretion before and 5 min after exercise in each group are shown in Figure 2. The Paired Samples T-test presented in Figure 2 displays no essential average UCP-1 secretion between before and 5 min after exercise on the AMIP group (P>0.05). In contrast, there is a significant difference in UCP-1 secretion before and 5 min after exercise on AHIP (P<0.05).

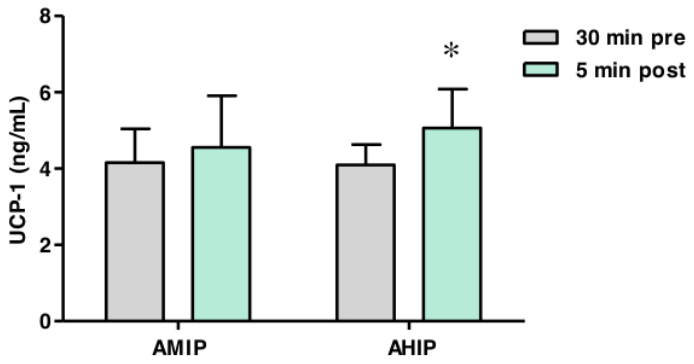

Figure 2. Mean of UCP-1 secretion analysis between before exercise and 5 min after exercise. (*) Significant with $P<0.05$ by Paired Samples T-test.

DISCUSSION

Exercise is one of the strategies and no-drug approaches to prevent the accelerating chronic disease prevalence, such as obesity, type 2 diabetes mellitus, and metabolic diseases (Dewal & Stanford, 2018). Therefore, exercise should be a lifestyle to prevent increasing chronic disease prevalence (Müller *et al.*, 2016), and regulate energy balance and body function (Garneau *et al.*, 2020). Chronic diseases and premature death will decrease significantly (Leal *et al.*, 2018). A molecular physiology study on exercise is required to counter the rise of chronic diseases.

Therefore, this research aims to analyze UCP-1 expression after high- and moderate-intensity acute exercise in young adults. The results demonstrate no difference in UCP-1 expression in both physical activities ($P>0.05$). Thirty minutes of high- and moderate-intensity acute exercise on young adults with excellent freshness levels have a similar response toward UCP-1 expression. A previous study by Kim *et al*. (2018), giving 30 minutes of different exercise intensity on the treadmill to lab animals, also reveals no significant difference in UCP-1 expression.

In addition, research using obese white Wistar rat lab animal given high-intensity exercise on a treadmill with 85%-90% maximum speed, along with continuous moderate-intensity with 65%-70% maximum speed toward Adipogenesis and Browning on adipose tissue reports no significant increase of UCP-1 on both groups (Khalafi *et al*., 2020b). Previous research also finds that exercise enhances UCP-1 expression and sympathetic nerve stimulation (Dewal and Stanford, 2018). In addition, the results of this study also revealed accelerated UCP-1 secretion occurred in both groups. The develop UCP-1 is mediated by the raise of gen 1-alpha (PGC-1α) periferisome proliferator-activated gen receptor activation on human skeletal muscle triggered by exercise (Reisi *et al*., 2016). PGC-1α is a co-transcription regulator that promotes various transcription managing complex gene regulatory networks that engage in the mitochondrial tissue content control producing the brown adipose tissue (BAT) production, while PGC-1α improves protein with five fibronectin type III 5 (FNDC5) domain that is divided and released to blood flow as irisin (Dinas *et al*., 2017).

The results of this research present that high-intensity exercise produces more extensive UCP-1 expression than moderate-intensity activity. The exercise intensity affects the increase of muscle contraction and accelerates sympathetic nerve stimulation (Aldiss *et al*., 2018). The intensively developed muscle construction during the exercise drives the metabolism escalation that inspires the advance of PGC-1α, which is higher on the high-intensity than the moderate-intensity (Shirvani and Arabzadeh, 2020). PGC1-α is the mediator of energy metabolism programming. It

controls mitochondrial biogenesis and oxidative metabolism, such as cell types, that stimulate UCP-1 expression (Dinas *et al*., 2017), in chronic and acute physical activities (Gorski *et al*., 2018). The accelerated UCP-1 expression comes from physical activity provocation mediated by co-activator PGC1α, increasing FNDC5 in muscle, secreted irisin, that boosts thermogenesis gene stimulant on adipocytes. Irisan functions as the signal administrator of energy secretion from the muscle directly communicated with adipose tissue and causes the browning, accelerating the metabolic profile of white adipose tissue (WAT) to improve the whole body energy release (Qiu *et al*., 2018).

In addition, adipose tissue is a dynamic tissue for energy metabolism, while brown adipose tissue operates as active tissue metabolically contributes toward thermogenesis through activating UCP-1 (Khalafi *et al*., 2020b). On the other hand, thermogenetic is commanded by the hypothalamus. Thus, body temperature shift leads to thermosensory stimulating dorsomedial hypothalamic as an adaptive thermogenesis control mediated by the hypothalamus (Morrison 2016). A study of rats exposed to cold reports that thermos sensory input enforced through the preoptic median prompts the dorsomedial hypothalamus (DMH) so that the sympathetic nerve activity is increased (Fuller-Jackson and Henry, 2018).

The increased catecholamine secretion may affect the escalated UCP-1 expression during the high-intensity exercise (Sanchez-Delgado *et al*., 2015). The adrenergic receptor captures the extension of catecholamine secretion, increasing cyclic adenosine monophosphate and then activating protein kinase A, drives lipolysis and UCP-1 secretion (Rodrigues *et al*., 2018). Noradrenaline binds β3, α1, and α2-adrenergic receptors. It generates the upsurge signal production that disturbs the process of lipolysis, thermogenesis, and gene expression. This way, β3 on BAT improves metabolism that activates UCP-1. UCP-1 is a non-related protein in mitochondria responsible for proton translocation through the respiratory chain producing heat. Besides, a continuous β-adrenergic stimulant from BAT produces thermogenic activity that develops UCP-1 expression and

BAT hyperplasia accountable for energy balance maintenance (de Queiroz *et al*., 2012) and decreasing reactive oxygen species (ROS) (Brondani *et al*., 2012).

A more intensive skeletal muscle construction influences the high degree of UCP-1 in high-intensity activity than in moderate-intensity exercise (Huh, 2018). The muscle construction intensity affects the increase of energy necessity, oxygen uptake, and more active AMPK, improving the active PGC-1α and the developed thermogenesis (Khalafi *et al*., 2020b). Irisin discharge begins the expansion of mitochondrial content and UCP-1 expression within white adipose tissue (WAT), which improves thermogenic (Mai *et al*., 2020). Energy expenditure also induces PGC-1α transcription muscle regulation, stimulates the FNDC5 protein-membrane increase, improves iron discharge, creates biogenesis in mitochondria, and UCP-1 expression, accelerates heat production, and increases energy expenditure (Moienneia and Attarzadeh Hosseini, 2016). It is proven that the energy expenditure profile during high-intensity exercise is more comprehensive than moderate-intensity, even without an essential different temperature increase (Sugiharto *et al*., 2019a).

Additionally, the developed muscle construction intensity on high-intensity exercise accelerates higher energy requirement and oxygen need, creating the surge of sympathetic nerve stimulant to free catecholamines, affecting UCP-1 expression that browning in fulfilling the energy demand (Dewal and Stanford, 2018). Previous research reports higher heart rate and blood pressure escalation in high-intensity than moderate-intensity exercise (Sugiharto *et al*., 2019b). Intense muscle contraction during high-intensity exercise is one of the factors that affect sympathetic nerve stimulus and catecholamines secretion that binds the β-adrenergic receptor, along with G protein; it drives the increase of adenylate cyclase activity, AMP cyclic (cAMP) activation, A-kinase protein, and p38MAPK, stimulates lipolysis that activates UCP-1 (Sanchez-Delgado *et al*., 2015). Research results reveal that intense skeletal muscle contraction during high-intensity exercise is the primary determinant of different UCP-1 expressions, even

when the UCP-1 secretion in the second group is not critically different. There is a possibility that the exercise duration, types of muscle contraction, and excellent subjects' freshness level have not significantly accelerated UCP-1 secretion. The exercise duration provides an authentic contribution toward UCP-1 expression. The research presents that 30 minutes of exercise with 60%-70% VO2max intensity provides no significant UCP-1 expression (Kim *et al.*, 2018). Therefore, future research should pay attention to exercise duration, different activity modifications, and additional browning markers related to UCP-1 levels, such as IL-6.

CONCLUSION

Exercise. The results of the study showed no difference in the expression of UCP-1 between pre and post-test in MIE. In contrast, the UCP-1 of pre and post-test on AHIP exhibits significant differences. The 30 minutes acute high-intensity exercise substantially accelerates the expression of UCP-1 than a severe moderate-intensity exercise in young adults. Thus, high-intensity exercise is proposed to improve UCP-1 expression for young adults and trained groups by still observing the level of training, heart rate, and blood pressure during the exercise. In contrast, untrained people are not suggested to use the high-intensity exercise. Besides, future researchers are expected to analyze untrained or obesity groups with different activity models.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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