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Irisin serum increasing pattern is higher at moderate-intensity continuous exercise than at moderate-intensity interval exercise in obese females

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RESEARCH ARTICLE

Abstract

Lifestyle, unhealthy eating patterns, and low physical activity become trigger factors of obesity. Therefore, lifestyle modification with an exercise-based nonpharmacological approach is one of the strategies for combat obesity. This study aims to analyse the response of moderate-intensity interval and continuous exercise to irisin level increasing pattern on the obese female. A total of 21 obese females were enrolled in this study and given moderate-intensity interval exercise (MIIE) and moderate-intensity continuous exercise (MICE). ELISA was used to quantify the serum level of irisin in all samples. Statistical analysis was performed using one way-ANOVA and Tukey's honestly significant difference (HSD) post hoc test. Mean irisin levels of pre-exercise at control (CON), MIIE, and MICE were 3.26 ± 1.28 , 3.44 ± 0.56 and 3.89 ± 1.08 ng/ml, respectively ($P=0.519$). The mean irisin level of 10 min post-exercise was 2.99 ± 0.86 ng/ml at CON, 4.82 ± 1.01 ng/ml at MIIE, and 5.99 ± 1.27 ng/ml at MICE ($P=0.000$). The mean irisin levels of 6 h post-exercise were 3.04 ± 0.60 , 4.56 ± 0.87 , and 5.73 ± 1.02 ng/ml at CON, MIIE, and MICE, respectively ($P=0.000$). The mean irisin level of 24 h post-exercise was 3.04 ± 0.91 ng/ml at CON, 4.64 ± 0.69 ng/ml at MIIE, and 5.69 ± 1.53 ng/ml at MICE ($P=0.002$). We conclude that the post-exercise serum irisin level increased in both MICE and MIIE subjects, and the post-exercise serum irisin level maintained higher in the MICE than in the MIIE in the obese female subjects.

Keywords: obesity, interval exercise, continuous exercise, treadmill, irisin pattern

1. Introduction

Obesity prevalence in 2015 reached 12% or equal to 603.7 million adults around the world (The Global Burden of Disease (GBD) 2015 Obesity Collaborators, 2017). The Southeast Asia prevalence was counted 1.7% in 1980 and increased to 6.2% in 2015 (Chooi *et al.*, 2019), while Riset Kesehatan Dasar (Riskesdas) in 2018 showed that the prevalence of obesity over 18 years old in Indonesia was 21.8%. This data was higher than that of 2013 (14.8%) and 2007 (10.5%) (Riskesdas, 2018). It is estimated that in 2030 prevalence will reach 57.8% of the world's population (Kelly *et al.*, 2008). Obesity prevalence tends to increase both

in developed and developing countries (Ng *et al.*, 2014; Norheim *et al.*, 2014) and becomes a serious threat to the world health (Akter *et al.*, 2014; Chooi *et al.*, 2019; Gadde *et al.*, 2018; Peterson *et al.*, 2014; Tsuchiya *et al.*, 2014).

Obesity is a disease with a high risk of complications, disability and early death (Akter *et al.*, 2014; Rosella *et al.*, 2019). Moreover, obesity increases the risk of cardiovascular disease (Ng *et al.*, 2014), type 2 Diabetes mellitus (Gadde *et al.*, 2018) and some cancers (Nimptsch *et al.*, 2019), hypertension, stroke (Agofure, 2017), gallstones, osteoarthritis (Bales and Buhr, 2008), dyslipidaemia, non-alcoholic fatty liver disease (NAFLD) (Moreno-Navarrete and Fernández-Real, 2019),

cerebrovascular disease, respiratory disease, gastrointestinal system, chronic kidney disease (Malnick and Knobler, 2006), and low life expectancy (Nimptsch *et al.*, 2019). Life style, unhealthy eating patterns, and low physical activity become trigger factors of obesity (Bautista *et al.*, 2019; Norheim *et al.*, 2014). Therefore, lifestyle modification with an exercise-based nonpharmacological approach is the right strategy (Murawska-Cialowicz *et al.*, 2015). Exercise is considered a very effective and efficient method of preventing obesity prevalence increment (Boström *et al.*, 2012; Huh *et al.*, 2014).

Exercise has increased the energy expenditure which is mediated by the irisin hormone (Tsuchiya *et al.*, 2014). Exercise induces irisin by activating peroxisome proliferation-activated receptor γ coactivator-1 α (PGC-1 α) (Boström *et al.*, 2012). PGC-1 α activation stimulates fibronectin type III domain-containing protein 5 (FNDC-5) expression (Fatouros, 2018) which induces irisin release to the blood circulation (Moreno-Navarrete *et al.*, 2013). The release of irisin will trigger a browning process on white adipose tissue by stimulating uncoupling protein-1 (UCP-1) gene expression via p38 mitogen-activated protein kinase (p38-MAPK) signalling, and increasing energy expenditure and decreasing lipid accumulation (Boström *et al.*, 2012; Fatouros, 2018; Perakakis *et al.*, 2017). Some studies have reported different results, due to irisin increment which did not occur at the same time and depending on the intensity of exercise (Huh *et al.*, 2014; Tsuchiya *et al.*, 2014; Winn *et al.*, 2017). The research by Huh *et al.* (2014) concluded that high-intensity interval training (HIIT) may lead to increases of acute response of plasma irisin levels 5 min post-intervention, more than continuous moderate-intensity exercise (CME) of males and females with a normal body mass index (BMI). Research by Tsuchiya *et al.* (2014) has shown that high-intensity exercise (HIE) causes an increase of irisin response after 6 and 19 h post-intervention compared to low-intensity exercise (LIE) on normal BMI males. The study by Winn *et al.* (2017) described that moderate-intensity continuous aerobic exercise (ModEx) increases irisin level during intervention and 190 min post-recovery compared to high-intensity aerobic interval exercise (IntEx) on female subjects with a BMI over 30 kg/m². According to those studies, no one has yet studied irisin secretion increment as a result of moderate-intensity interval exercise (MIIE) and moderate-intensity continuous exercise (MICE) conducted in the morning by female teenagers aged 18-23 years with a BMI of 25-35 kg/m² and percentage body fat (PBF) over 30%. These results on irisin secretion pattern and exercise intensity are important to support future exercise intervention strategies against obesity.

The aim of this study was to determine the effect of MICE and MIIE on irisin levels in obese females. We hypothesised that the greater metabolic stress induced by MICE would enhance higher peak serum irisin levels compared with MICE.

2. Materials and methods

Experimental design

This study was a true experiment using a basic time series design. The subjects were 21 obese females aged 18-23 years with a BMI of 25-35 kg/m², PBF over 30%, normal blood pressure, normal resting heart rate (RHR), normal oxygen saturation (SpO₂) of 95-100%, fasting blood glucose (FBG) under 100 mg/dl and normal haemoglobin (Hb). They were randomly divided into three groups, CON (n=7, control group without intervention), MIIE (n=7), and MICE (n=7). All subjects received verbal or written information about the research. Subjects filled out and signed an informed consent before participating in the study. All procedures were approved by The Health Research Ethics Committee Faculty of Medicine Universitas Airlangga Surabaya no. 309/EC/KEPK/FKUA/2019.

Exercise protocol

MIIE intervention was performed by using a moderate-intensity treadmill with 60-70% HR_{max} for 45 min, which is divided into 5 min warming up (50-60% HR_{max}), 35 min core exercise (5 min workout (60-70% HR_{max}) inserted by an active recovery above treadmill tool for 2.5 min (50-60% HR_{max}), repeated 5 times) and 5 min cooling down (50-60% HR_{max}). Moderate-intensity continuous exercise (MICE) intervention was done by running on a treadmill tool with 60-70% HR_{max} intensity for 40 min, which was divided into 5 min warming up (50-60% HR_{max}), 30 min core exercise continuously (60-70% HR_{max}) and 5 min cooling down (50-60% HR_{max}) (Dias *et al.*, 2018; Garber *et al.*, 2011; Tew *et al.*, 2019; Wewege *et al.*, 2017). This experiment was performed between 07:00-09:00 AM by using a treadmill (Richter Treadmill Semi-Commercial Evolution (4.0HP DC); Richter Fitness, Taipei, Taiwan). Further information about the duration and intensity scheme of MIIE and MICE is shown in Figure 1.

Blood collection

Blood samples were obtained from a cubital vein of 4 ml (Daskalopoulou *et al.*, 2014). It was taken four times (pre-exercise, 10 min, 6 h, and 24 h post-exercise). The blood samples were centrifuged for 15 min at a speed of 3,000 rpm. The serum was separated and saved at -80 °C until analysis on the next day (Daskalopoulou *et al.*, 2014; Tsuchiya *et al.*, 2014, 2015).

Blood analysis

Irisin level was measured by a commercial ELISA kit (EK-067-29; Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) with a standard curve range of 1.9-1000 ng/ml and irisin sensitivity level of 1.9 ng/ml. FBG was measured in

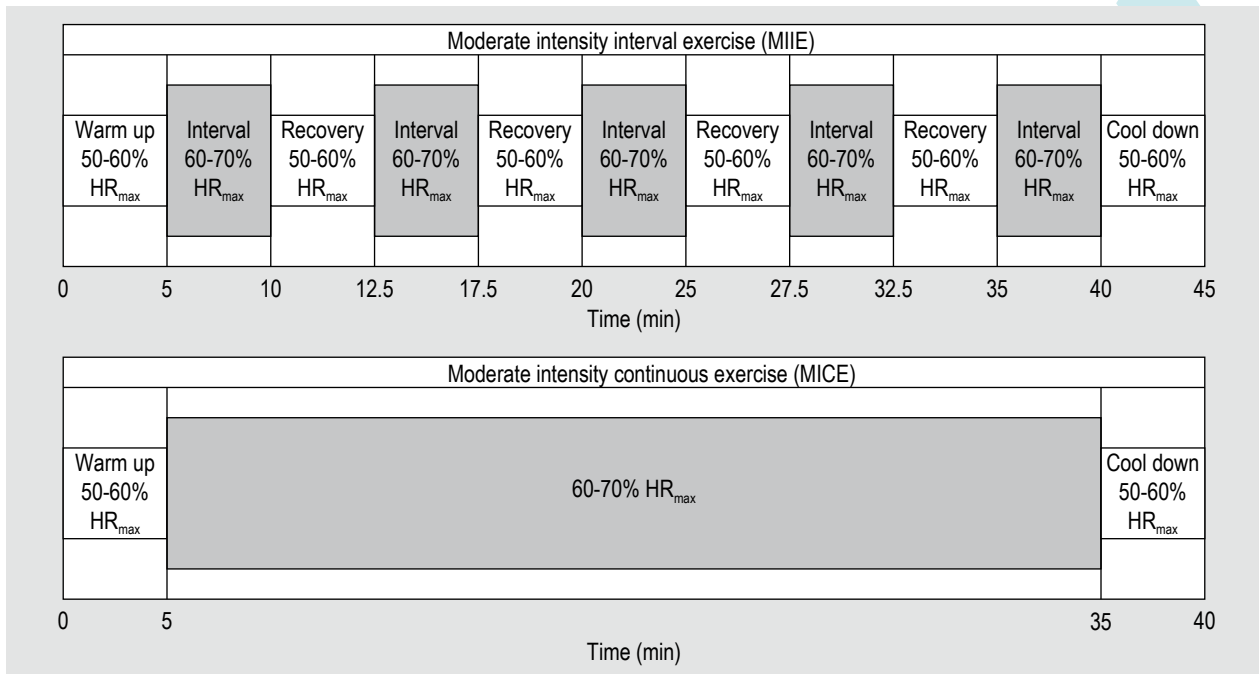


Figure 1. Scheme of detailed duration and intensity of moderate-intensity interval exercise (MIE) and moderate-intensity continuous exercise (MICE). HR_{max} = maximum heart rate.

mg/dl using an Accu-Chek Performa (Roche, Mannheim, Germany), while Hb was measured g/dl by Easy Touch GCHb (Easy Touch, Hsinchu, Taiwan).

Body composition analysis

The body height of the subject was measured using a stadiometer (SECA, Chino, CA, USA). Body composition includes of body weight, BMI, PBF, fat mass, free fat mass, muscle mass, total body water, bone mass and basal metabolic rate were measured by bio-impedance analysis using a TANITA Body Composition Analyzer DC3607601(2)-1604 FA (TANITA Corporation of America, Inc., Arlington Heights, IL, USA). Waist circumference (WC) was measured by wrapping anthropometric tape measure circularly on the middle between the lower rib and iliac which was parallel to the midaxillary line, whereas hip circumference (HC) was measured by wrapping anthropometric tape measure circularly on great trochanter (Vatier *et al.*, 2014). Waist to hip ratio (WHR) was counted WC divided by HC. This body composition measurement was done at 24 h pre-intervention.

Maximal oxygen volume test

Measurement of maximal oxygen volume (VO_{2max}) by the Astrand 6-min cycle test method was performed by using a Monark 828 E Version 1010 ergo cycle (Monark, Vansbro, Sweden). The heart rate was monitored by a Polar H10 Heart Rate Sensor (Polar Electric, Inc., Bethpage, NY, USA), while oxygen saturation (SpO₂) was evaluated by using a

Beurer Pulse Oximeter (PO 30 Pulse Oximeter, Beurer North America LP, Hallandale Beach, FL, USA). Blood pressure was measured using an OMRON automated device (OMRON Model HEM-7130 L, Omron Co., Osaka, Japan) at the nondominant arm 3 times consecutively with a 1-2 min interval between two measurements while participants were in a seated position.

Statistical analysis

Data were analysed using SPSS for Windows, version 16 (SPSS Inc., Chicago, IL, USA). The normality of data was tested using Shapiro-Wilk, whereas homogeneity was checked using the Levene test. A comparison test was done using one-way ANOVA, two-way ANOVA and continued by Tukey's honestly significant difference (HSD) post hoc test. All data were presented as mean ± standard deviation (SD) and $P < 0.05$ was considered significant.

3. Results

Descriptive analysis of subjects' characteristics (anthropometry, physical and physiological condition) in each group is presented in Table 1. The result of the one-way ANOVA test concluded that there was no differences in the subjects' characteristics on all variables from each group ($P > 0.05$). Analysis result of pre-exercise, 10 min post-exercise, 6 h post-exercise, and 24 h post-exercise irisin level is shown in Table 2. It shows that the irisin level of MICE is higher than that of MIE and CON in all-time recorded post-exercise.

Table 1. Subject baseline characteristics.¹

Variable	CON (n=7)	MIIE (n=7)	MICE (n=7)	ANOVA P-values
Anthropometry				
Age (years)	20.67±1.03	21.29±1.49	20.71±0.76	0.551
Body weight (kg)	75.23±6.74	73.26±8.83	72.60±7.89	0.829
Body height (m)	1.59±0.05	1.58±0.07	1.57±0.05	0.942
Body mass index (kg/m ²)	29.85±1.60	28.97±1.85	29.11±1.47	0.606
Percentage body fat (%)	45.47±3.16	43.79±2.43	44.29±2.68	0.546
Fat mass (kg)	34.18±4.14	32.24±4.89	32.33±5.09	0.720
Free fat mass (kg)	40.92±4.25	41.20±4.74	40.33±3.21	0.922
Muscle mass (kg)	38.52±3.89	38.76±4.33	37.96±2.93	0.921
Total body water (kg)	30.20±4.33	31.01±3.86	30.67±2.32	0.919
Total body water (%)	40.15±3.50	42.30±2.24	42.41±2.86	0.315
Bone mass (kg)	2.40±0.35	2.44±0.40	2.37±0.28	0.929
Basal metabolic rate (kcal)	1,366.17±136.62	1,363.29±154.79	1,342.86±108.48	0.942
Waist circumference (cm)	88.17±6.49	85.86±8.33	87.29±10.34	0.888
Hip circumference (cm)	109.33±3.56	107.29±7.89	107.86±6.62	0.843
Waist to hip ratio	0.81±0.03	0.79±0.04	0.80±0.05	0.771
Physical condition				
Resting heart rate (bpm)	76.33±11.27	85.14±10.91	77.86±8.99	0.278
Maximum heart rate (bpm)	199.33±1.03	199.29±1.38	199.43±0.79	0.970
VO _{2max} (ml/kg/min)	27.41±2.37	26.17±1.04	27.51±1.27	0.260
Physiological condition				
Systolic blood pressure (mmHg)	113.33±5.16	112.86±4.88	111.43±3.78	0.739
Diastolic blood pressure (mmHg)	76.67±5.16	75.71±5.34	74.29±5.34	0.719
Oxygen saturation (%)	98.17±0.75	97.86±0.89	98.00±0.82	0.801
Body temperature (°C)	36.32±0.69	36.23±0.49	35.93±0.75	0.537
Fasting blood glucose (mg/dl)	91.67±4.55	89.57±6.13	89.57±8.16	0.809
Hemoglobin (g/dl)	15.32±1.99	15.21±1.01	14.53±1.11	0.544

¹ Data are represented as Mean ± standard deviation. CON = control group; MIIE = moderate-intensity interval exercise group; MICE = moderate-intensity continuous exercise group.

Table 2. Irisin level in each group based on time blood samples.^{1,2}

Time	n	CON (ng/ml)	MIIE (ng/ml)	MICE (ng/ml)	ANOVA P-values
Pre-exercise	7	3.26±1.28	3.44±0.56	3.89±1.08	0.519
10 min post-exercise	7	2.99±0.86	4.82±1.01*	5.99±1.27*	0.000
6 h post-exercise	7	3.04±0.60	4.56±0.87*	5.73±1.02*†	0.000
24 h post-exercise	7	3.04±0.91	4.64±0.69*	5.69±1.53*	0.002

¹ Data are represented as Mean ± standard deviation. CON = control group; MIIE = moderate-intensity interval exercise group; MICE = moderate-intensity continuous exercise group. P-values were obtained using one-way ANOVA.

² Tukey's HSD post hoc test was used to compare irisin levels between groups. 10 min post-exercise (*) significant vs CON ($P \leq 0.05$). 6 h post-exercise (*) significant vs CON ($P \leq 0.05$) and (†) significant vs MIIE ($P \leq 0.05$). 24 h post-exercise (*) significant vs CON ($P \leq 0.05$).

According to the one-way ANOVA test, the pre-exercise mean irisin level in all groups is not significantly different ($P=0.519$), whereas at 10 min post-exercise the mean irisin level showed a significant difference ($P=0.000$). There are also significant differences at 6 h ($P=0.000$) and 24 h post-exercise ($P=0.002$). Based on the Tukey's HSD post hoc test, there is a significant difference of 10 min post-

exercise irisin level between MIIE and CON ($P=0.018$), MICE and CON ($P=0.000$), while MIIE and MICE are not significantly different ($P=0.131$). At 6 h post-exercise, the irisin level was significantly different between MIIE and CON ($P=0.014$), MICE and CON ($P=0.000$), and MICE and MIIE ($P=0.042$). A significant difference is also found at 24 h post-exercise between MIIE and CON ($P=0.048$),

MICE and CON ($P=0.001$), but not between MIE and MICE ($P=0.211$).

Figures 2-4 provide a graphical representation of the mean irisin level in each group. Figure 2 shows the result of the two-way ANOVA test indicating there is no significant difference in irisin level based on time samples taken in CON ($P>0.05$), whereas MIE and MICE shows a significant difference in irisin level based on time samples ($P<0.05$) (Figure 3 and 4). Tukey HSD post hoc test of MIE shows a significant difference in irisin level between 10 min post-exercise and pre-exercise ($P=0.018$), between 6 h post-exercise and pre-exercise ($P=0.047$), also between 24 h post-exercise and pre-exercise ($P=0.046$). Meanwhile, there are no significant differences between 10 min post-exercise and 6 h post-exercise ($P=0.929$), 10 min post-exercise and 24 h post-exercise ($P=0.974$), as well as 6 h post-exercise and 24 h post-exercise ($P=0.998$) (Figure 3). Based on Tukey HSD post hoc test MICE showed a significant difference in irisin level between 10 min post-exercise and pre-exercise ($P=0.020$), 6 h post-exercise and pre-exercise ($P=0.038$), and 24 h post-exercise and pre-exercise ($P=0.045$). Conversely, there are no significant differences in irisin level between 10 min post-exercise and 6 h post-exercise ($P=0.979$), 10 min post-exercise and 24 h post-exercise ($P=0.968$), as well as 6 h post-exercise and 24 h post-exercise ($P=1.000$) (Figure 4).

4. Discussion

This study was conducted to analyse the response of MIE and MICE to the increment of irisin levels in females with obesity. According to the one-way ANOVA test, the subjects' characteristics (anthropometry, physical and

physiological condition) in all groups were similar ($P>0.05$). Therefore, the three groups were in the same starting point of pre-intervention.

The female subjects were selected based on the risk level of obesity. Females have a higher risk level of overweight and obesity by 1.76 and 3.43 times, respectively, than males (Sudikno *et al.*, 2015). The diagnosis used to determine

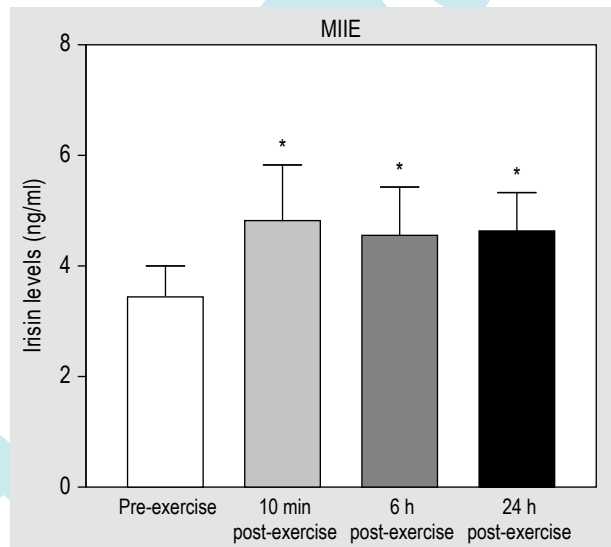


Figure 3. Mean irisin level of the moderate-intensity interval exercise group. Data are shown as mean \pm standard deviation. P -values were obtained using two-way ANOVA to compare 10 min, 6 h, 24 h post-exercise with pre-exercise irisin levels. * Significant vs pre-exercise ($P\leq 0.05$).

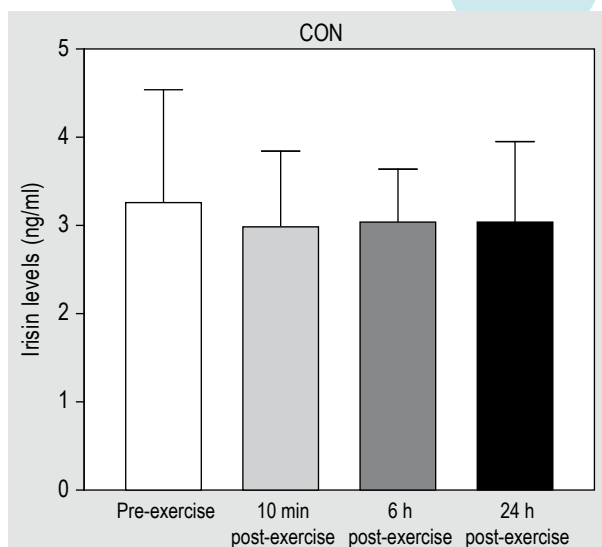


Figure 2. Mean irisin level of the control group. Data are shown as mean \pm standard deviation. P -values were obtained using two-way ANOVA to compare 10 min, 6 h, 24 h post-exercise with pre-exercise irisin levels.

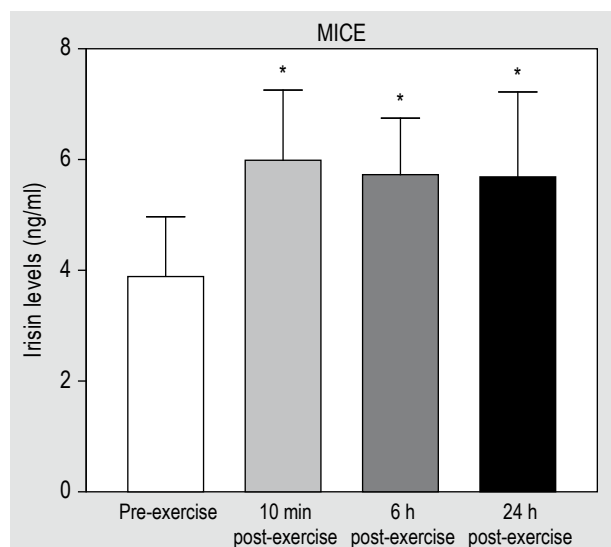


Figure 4. Mean irisin level of the moderate-intensity continuous exercise group. Data are shown as mean \pm standard deviation. P -values were obtained using two-way ANOVA to compare 10 min, 6 h, 24 h post-exercise with pre-exercise irisin levels. * Significant vs pre-exercise ($P\leq 0.05$).

obesity was by anthropometric methods (Sudargo *et al.*, 2016), such as BMI, skinfold thickness, WHR, bioelectrical impedance analysis (BIA) and dual-energy x-ray absorptiometry (DEXA) (Beechy *et al.*, 2012; Da Silva *et al.*, 2016; Sudargo *et al.*, 2016; Visscher *et al.*, 2010; Zeng *et al.*, 2012). However, this study only used the BMI and PBF parameters to describe body composition in diagnosing obesity. BMI is a parameter used to detect obesity and measure body weight over time (Beechy *et al.*, 2012). However, BMI has low accuracy caused by the inability to describe human composition, such as free fatty mass, fat mass and body fat distribution (Akpınar *et al.*, 2007). Besides, BMI cannot distinguish between fat mass and muscle mass (Nimptsch *et al.*, 2019). Therefore, only using BMI as measurement of body composition to determine the obesity level is not recommended, as it will inhibit obesity prevention and control in the future (Beechy *et al.*, 2012; Chooi *et al.*, 2019). PBF is a body component beside bone mass, muscle mass and body water. PBF represents the body fat mass proportion in body weight (Zeng *et al.*, 2012). Females possess a natural higher PBF than males (Blaak, 2001); they tend to have a 10% higher PBF than that of males in the same BMI class (Jackson *et al.*, 2002). An individual can be classified as obese if the PBF $\geq 25\%$ (males) or $\geq 30\%$ (females), according to the Asian BMI criteria (Wen *et al.*, 2009); thus a PBF over 30% was used to classify obesity in this study.

There was a significant difference in irisin level at 10 min, 6 h dan 24 post-exercise in the three groups. We found that both MIIE and MICE had increasing irisin levels at all recorded post-exercise times compared to pre-exercise in each group. During exercise, there was an increase in energy demand for muscle contraction, thus the energy reserved in the muscle decreased, and consequently, increased the release of irisin into the blood to maintain energy balance during exercise. Huh *et al.* (2012) stated that irisin levels significantly increased when muscle ATP decreases. Previous studies conducted on healthy human subjects suggested that irisin and lactate concentrations were positively correlated with increasing exercise load (Daskalopoulou *et al.*, 2014). Our result is similar to the previous researcher's hypothesis that increment of irisin release correlates with energy demands of the muscle during contraction (Daskalopoulou *et al.*, 2014). Our result indicates that the increase in muscle contraction and decrease in ATP during exercise becomes one of marking factors in the increment of irisin release to the blood circulation (Maalouf and Khoury, 2019). Exercise induces irisin release via peroxisome proliferator-activated receptor- γ (PPAR- γ) and PGC-1 α (Spiegelman, 2013). PPAR- γ and PGC-1 α are multispecific transcriptional co-activators that regulate genes as a response to the nutritional and physiological signals of the tissue. PPAR- γ and PGC-1 α are expressed in skeletal muscle, brown adipose tissue, liver and heart (Gizaw *et al.*, 2017; Moreno-Navarrete *et al.*,

2013; Norheim *et al.*, 2014; Xu, 2013). Moderate-intensity exercise increases PGC-1 α activation, especially in the heart and skeletal muscle, and also increases some metabolic parameters, such as insulin sensitivity and signalling, and supports AMPK activation, PGC-1 α phosphorylation and FNDC5 production; this is followed by FNDC5 division to produce irisin, which will be released into the blood (Moreno-Navarrete *et al.*, 2013; Norheim *et al.*, 2014; Xu, 2013).

According to the analysis, the irisin level increase in MICE was higher than in MIIE and CON. This is similar to the study conducted by Winn *et al.* (2017) using female subjects aged 18-35 years with a BMI over 30 kg/m² performing moderate-intensity aerobic exercise (ModEx). The study showed that ModEx significantly increased irisin levels, both during intervention and 190 min post-recovery. It is similar to the study conducted by Kraemer *et al.* (2014) using female and male teenager subjects, who had been instructed to do MICE (60% VO_{2max}) using a treadmill. Their results showed that MICE significantly increased irisin levels about 20.4% for males and 24.6% for females. The study by Huh *et al.* (2014) reported that moderate-intensity exercise (CME) significantly increased irisin levels in healthy males. Irisin level increment in MICE is caused by a higher energy requirement than MIIE, thus PGC-1 α is activated. PGC-1 α activation triggers FNDC-5 expression (Fatouros, 2018) and induces proteolytic cleavage of the FNDC-5 membrane protein in skeletal muscle. Consequently, irisin will be released to the blood circulation (Moreno-Navarrete *et al.*, 2013). The release of irisin into the blood stimulates the browning process in white adipose tissue by triggering UCP-1 expression via p38-MAPK signalling. The process causes an increase of energy expenditure and a decrease of lipid accumulation (Boström *et al.*, 2012; Fatouros, 2018; Perakakis *et al.*, 2017).

Irisin is a hormone that modulates the effect of exercise by regulating energy expenditure and lipid oxidation (Boström *et al.*, 2012). Irisin may act as a muscle-derived energy-expenditure signal that directly communicates with adipose tissue and induces the browning process (Pardo *et al.*, 2014). This effect may improve the white adipose tissue metabolic profile and enhance whole-body energy expenditure, making irisin a potential new target for the treatment of metabolic diseases, including obesity (Benedini *et al.*, 2017; Pardo *et al.*, 2014). Although irisin is known as an exercise-induced myokine, many studies showed inconsistent results. Moreover, highly controversial results concerning the effects of exercise on irisin have been also reported. Based on the systematic review conducted by Dinas *et al.* (2017), thirteen studies showed that acute exercise increased circulating irisin in healthy individuals, while five studies showed no effect of acute exercise on circulating irisin. The study by Huh *et al.* (2012) reported a significant difference in irisin level by acute exercise in

a healthy male, whereas Kurdiova *et al.* (2014) reported that exercise, in an acute form applied to either sedentary or trained individuals, was not effective in modulating the irisin circulating levels and skeletal muscle FNDC5 gene expression. Khodadadi *et al.* (2014) reported that high-intensity interval acute exercise increased irisin (33%, $P=0.039$) in overweight females. Löffler *et al.* (2015) also reported that acute exercise increased irisin levels in both adults ($P=0.006$) and children ($P<0.001$). However, Aydin *et al.* (2013) reported no changes in serum irisin after acute exercise in obese and normal weight males. Moienneia and Hosseini (2016) also found an unchanged irisin level after acute exercise for both low- and high-intensity exercise in sedentary young healthy females. These differences might be the result of a different set-up of the studies compared to our experiments, such as (1) the subjects (they recruited sedentary young healthy females, whereas we recruited young obese females), (2) the intensity of exercise (they applied low-intensity and high-intensity resistance training, whereas we applied MIIE and MICE with treadmill). Therefore, further studies are necessary to evaluate the effects of MIIE and MICE against increase irisin levels on an obese female with a similar study design.

Moreover, highly controversial results obtained with different ELISA kits in exercise studies have been reported. For instance, the research by Huh *et al.* (2015) which compared the resistance exercise results, showed the most dynamic irisin changes (measured using the EK-067-52 kit, Phoenix Pharmaceuticals), with our results using the EK-067-29 kit. Results with the EK-067-52 kit showed approximately 10-fold higher irisin levels than with the EK-067-29 kit, and there was a low correlation between the results from the two kits ($r=0.233$, $P=0.191$). Furthermore, Kurdiova *et al.* (2014) reported poor agreement between ELISA kit RK-067-16 (Phoenix Pharmaceuticals) and EK-067-29. Similarly, Montes-Nieto *et al.* (2016) analysed human irisin using two different lots (604824 and 605835) of the EK-067-29 ELISA kit and reported that serum irisin levels determined by lot 604824 were higher in patients with weight excess when compared with lean subjects, but this difference was not found when assaying irisin with lot 605835. Finally, Albrecht *et al.* (2015) also reported that irisin circulating in the blood is largely based on commercial ELISA kits which are based on polyclonal antibodies (pAbs) not previously tested for cross-reacting serum proteins. Albrecht *et al.* (2015) analysed four commercial pAbs by Western blotting, which revealed prominent cross-reactivity with non-specific proteins in human and animal sera. Using recombinant glycosylated and non-glycosylated irisin as positive controls, they found no immune-reactive bands of the expected size in any biological samples. A FNDC5 signature was identified at 20 kDa by mass spectrometry in human serum, but was not detected by the commercial pAbs tested (Albrecht *et al.*, 2015).

Limitations to this current study include (1) small sample size, (2) high drop-out rate, (3) analysis of body composition by BIA, (4) only one parameter measured, (5) lack of evidence of browning markers, and (6) this study only conducted an acute exercise. Firstly, in this study we only used a small sample size with the total number of subjects 21 obese females. Therefore, a future study should include a larger number of female obese subjects. Secondly, the high drop-out rate in each group was as one for the control group, three for MIIE and 5 for the MICE group. The high drop-out rate was due to the inability of the subject to follow the treadmill rhythm; thus the intervention was stopped and the subject was declared a failure in our study methods (dropout). Another subject was also found to be unable to continue to the blood sampling process, due to sickness with physical diagnosis of shortness of breath, pale face, dizziness, and a body temperature above 37.5 °C. Thirdly, although previous studies have reported that BIA has similarity with DEXA in measuring free fat mass, fat mass, PBF, and muscle mass (Fox *et al.*, 1996, Pratley *et al.*, 2000), BIA could not provide conclusive information of body composition that DEXA could have provided (Kim *et al.*, 2016). Fourthly, we only used one parameter to measure irisin levels, whereas other parameters, such as PGC-1 α and FNDC-5 would have provided information on irisin levels as well. Fifthly, we were unable to estimate browning markers, such as UCP1, PR domain containing 16 (PRDM16), and other cytokines that might be related to circulating irisin levels, such as interleukin 6. Lastly, this study only conducted an acute intervention (exercise), thus further studies are required to find out the effect of a chronic intervention (training) in obese females. Besides, irisin measurements should be done in more than 24 h (e.g. 36, 48, and 72 h post-exercise). The results are expected to be useful for stabilising an optimal exercise frequency in obesity management in the future.

5. Conclusions

Based on the results of this study it can be concluded that the post-exercise serum irisin levels increased both in MICE and MIIE, and post-exercise serum irisin level were maintained at a higher level in MICE compared to MIIE in obese females. These results suggest that MICE is more effective to increase serum irisin levels than MIIE.

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Conflict of interest

The authors declare that they have no competing interests.

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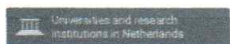
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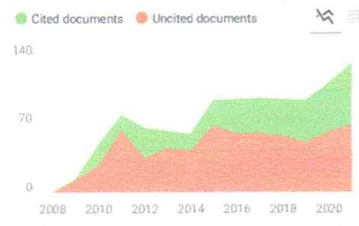
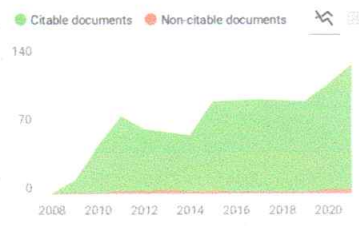
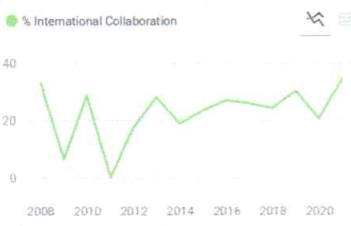
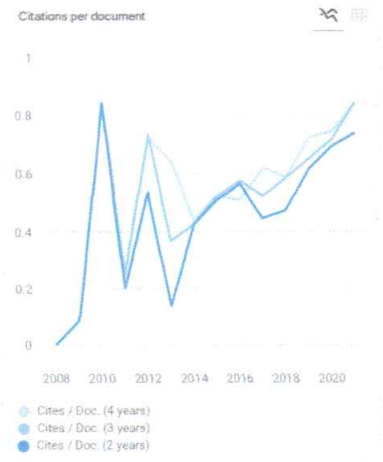
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