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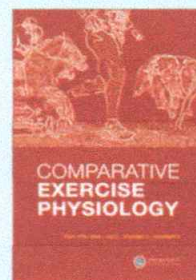
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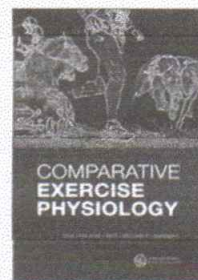
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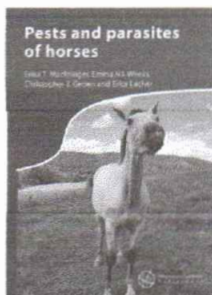
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Inflammatory markers in response to interval and continuous exercise in obese women

A. Andarianto¹, P.S. Rejeki^{1,2,3*}, Sakina^{2,4}, A. Pranoto⁵, T.W. Aga Seputra¹, Sugiharto⁶ and M. Miftahussurur^{7,8}

¹Sport Health Science, Faculty of Medicine, Universitas Airlangga, Prof. Dr. Moestopo No.47 Street, Pacar Kembang, Kec. Tambaksari, 60131 Surabaya, Indonesia; ²Medical Program, Faculty of Medicine, Universitas Airlangga, Prof. Dr. Moestopo No.47 Street, Pacar Kembang, Kec. Tambaksari, 60131 Surabaya, Indonesia; ³Physiology Division, Department of Physiology and Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Prof. Dr. Moestopo No.47 Street, Pacar Kembang, Kec. Tambaksari, 60131 Surabaya, Indonesia; ⁴Department of Anatomy, Histology, and Pharmacology, Faculty of Medicine Universitas Airlangga, Prof. Dr. Moestopo No.47 Street, Pacar Kembang, Kec. Tambaksari, 60131 Surabaya Indonesia; ⁵Medical Science, Faculty of Medicine, Universitas Airlangga, Prof. Dr. Moestopo No.47 Street, Pacar Kembang, Kec. Tambaksari, 60131 Surabaya, Indonesia; ⁶Department of Sport Science, Faculty of Sport Science, Universitas Negeri Malang, Jl. Semarang No.5, Sumbersari, Kec. Lowokwaru, 65145 Malang, Indonesia; ⁷Institute of Tropical Disease, Universitas Airlangga, Kampus C, Jl. Unair, Mulyorejo, Kec. Gubeng, 60286 Surabaya, Indonesia; ⁸Gastroentero-Hepatology Division, Department of Internal Medicine, Faculty of Medicine, Dr. Soetomo Teaching Hospital, Universitas Airlangga, Jalan Mayjen, Prof. Dr. Moestopo No. 6-8, 60286 Surabaya, Indonesia; purwo-s-r@fk.unair.ac.id

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RESEARCH ARTICLE

Abstract

Obesity is strongly associated with the degree of inflammation characterised by proinflammatory cytokines, such as tumour necrosis factor- α (TNF- α). Lifestyle modification with exercise is the right strategy because it can stimulate interleukin 6 (IL-6) secretion which acts as an anti-inflammatory. This study aimed to analyse the response of interval and continuous exercise to inflammatory markers in obese women. Twenty-four women participated in this study and were randomly divided into 3 groups: CONG (n=8, control group without any intervention); MCEG (n=8, continuous exercise group) and MIEG (n=8, interval exercise group). ELISA was used to measure the levels of IL-6 and TNF- α , pre-exercise and post-exercise. The data were analysed using the paired sample t-test. The mean levels of TNF- α , pre-exercise and post-exercise, were 19.35 ± 2.73 vs 19.36 ± 2.23 pg/ml ($P=0.989$) in CONG, 19.42 ± 2.79 vs 16.63 ± 0.82 pg/ml ($P=0.017$) in MCEG, and 19.46 ± 3.08 vs 16.96 ± 2.11 pg/ml ($P=0.079$) in MIEG. Mean levels of IL-6, pre-exercise and post-exercise, were 7.56 ± 2.88 vs 7.66 ± 4.12 pg/ml ($P=0.957$) for CONG, 7.68 ± 3.41 vs 13.97 ± 2.38 pg/ml ($P=0.001$) for MCEG, and 7.78 ± 1.99 vs 13.66 ± 3.55 pg/ml ($P=0.001$) for MIEG. We concluded that interval and continuous exercise decreased pro-inflammatory and increased anti-inflammatory cytokines.

Keywords: inflammatory markers, interval exercise, continuous exercise, obese women

1. Introduction

In 2016, more than 1.9 billion adults of 18 years and over were overweight and more than 650 million of them were obese (WHO, 2016). Obesity is more common in women than men (Rybnikova *et al.*, 2016). The increasing prevalence of obesity occurs in both developing and developed countries (Ng *et al.*, 2014; Norheim *et al.*, 2014). The prevalence of obesity in Indonesia in 2018 (21.8%) was higher than in 2013 (14.8%) (Basic Health Research, 2018). In addition, when social distancing was established due to the coronavirus disease (COVID-19) pandemic, more than

40% of American people were considered obese (body mass index (BMI) ≥ 30 kg/m²) (Kass, 2020). The high increase in obesity prevalence is a serious problem that will threaten the quality of human resources and increase health problems in many countries (Oto *et al.*, 2014).

Obesity is a complex multifactorial disease (Chooi *et al.*, 2019) and considered as risk factor for non-communicable diseases (NCDs), such as type 2 diabetes mellitus, hypertension, dyslipidaemia, heart disease, and osteoarthritis (Hruby and Hu, 2015) that can cause early death (Hawley and McGarvey, 2015). Obesity is also

systematically related to the level of inflammation and the risk of developing cancer cells (Heikkilä *et al.*, 2008). It is characterised by high levels of inflammatory markers, such as tumour necrosis factor- α (TNF- α) in obese persons (De Lorenzo *et al.*, 2016). Obesity can be genetic or caused by lifestyle (Rybnikova *et al.*, 2016), but lifestyle (unhealthy diet and low physical activity) is the main triggering factor for obesity (Hernández Bautista *et al.*, 2019; Yaya and Ghose, 2019). Therefore, lifestyle modification with exercise is the right strategy (Murawska-Cialowicz *et al.*, 2015); increasing the recruitment of skeletal muscles to do mechanical work when the body does exercise is believed to have an anti-inflammatory effect (Dantas *et al.*, 2019; Gleeson *et al.*, 2011; Pedersen, 2017; Schmidt *et al.*, 2015). One of the cytokines secreted by skeletal muscles during exercise is interleukin 6 (IL-6) (Muñoz-Cánoves *et al.*, 2013). This cytokine is a multifunctional immunoregulatory cytokine that plays a role in the immune system (Bashashati *et al.*, 2017) and activates T cells as immunotherapy related to inflammation (Xu *et al.*, 2017).

Exercise can significantly increase IL-6 levels both in plasma (Costello *et al.*, 2018; Cullen *et al.*, 2015; Ellingsgaard *et al.*, 2019), and in blood serum (Briken *et al.*, 2016; Deveci and Şanlıer, 2018; Luna *et al.*, 2011). This increase correlates with muscle contraction. Signalling of IL-6 also correlates with stimulation of muscle hypertrophy and myogenesis through regulation of the proliferative capacity of muscle stem cells (Muñoz-Cánoves *et al.*, 2013), as well as stimulating the secretion of anti-inflammatory cytokines, such as interleukin 1 receptor antagonist (IL1RA) in order to decrease pro-inflammatory cytokines (Benatti and Pedersen, 2015). This mechanism indirectly explains that exercise can prevent TNF- α increase mediated by IL-6 increase (Mauer *et al.*, 2014). However, several studies have reported controversial results. Research by Newlin *et al.* (2012) reported that treadmill exercise with an intensity of 65% VO_{2max} significantly increased IL-6 levels. Meanwhile, the results of Lira *et al.* (2017) showed that immediately after moderate-intensity treadmill exercise IL-6 levels will decrease. Research conducted by Bernecker *et al.* (2013) revealed that during a marathon race the TNF- α levels increase. However, research by Hoseini *et al.* (2018) reported that aerobic exercise with an intensity of 70% HR_{max} can reduce TNF- α levels. So far, the effect of exercise on obesity related to IL-6 and TNF- α parameters has not been explored. Therefore, this study aims to reveal the response of inflammatory markers as a result of moderate-intensity interval and continuous exercise in obese women aged 18-23 years with a BMI of 25-35 kg/m². The results of this study can be used as a strategy to reduce the prevalence and level of inflammation in obesity.

2. Material and methods

Experimental design

This study used the Randomized Pretest-Posttest Control Group Design with 24 obese women aged 19-23 years as subjects. The subjects had a body mass index (BMI) of 27-32 kg/m², maximal oxygen volume (VO_{2max}) 26-30 ml/kg/min examined using the Astrand 6-min cycle test, normal blood pressure (110/70 to 120/80 mmHg), normal resting heart rate (RHR) (60-80 bpm), fasting blood glucose (FBG) \leq 100 mg/dl, normal haemoglobin (Hb) (13-18 g/dl), normal oxygen saturation (SpO_2) (95-100%). The subjects were randomly divided into 3 groups, namely CONG (n=8, control group without intervention): MCEG (n=8, moderate-intensity continuous exercise group) and MIEG (n=8, moderate-intensity interval exercise group). All subjects received verbal or written information about the research. Subjects filled out and signed an informed consent before participating in the study. All research procedures have been approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Airlangga Surabaya, number 309/EC/KEPK/FKUA/2019.

Exercise protocol

Continuous exercise was done by running on a treadmill with an intensity of 60-70% HR_{max} for 40 min, and consisted of 5 min of warming up (50-60% HR_{max}), 30 min of core exercise (60-70% HR_{max}) and 5 min of cooling (50-60% HR_{max}). Interval exercise was done by running on a treadmill with moderate intensity 60-70% HR_{max} for 45 min, and consisted of 5 min of warm-up (50-60% HR_{max}), 35 min of core exercise (5 min of work at 60-70% HR_{max} interspersed with active recovery on a treadmill for 2.5 min at 50-60% HR_{max} in 5 repetitions) and 5 min of cooling down (50-60% HR_{max}) (Dias *et al.*, 2018; Norton *et al.*, 2010; Rejeki *et al.*, 2021; Wewege *et al.*, 2017). Continuous intervention and interval exercise were performed at 7:00-09:00 am (Kraemer *et al.*, 2014; Nygaard *et al.*, 2015) using a Pulsar 4.0 HP Cosmos Sports & Medical treadmill (Nussdorf-Traunstein, Germany) (Nedić *et al.*, 2017). During exercise, heart rate was monitored using a polar heart rate monitor (Polar H10 Heart Rate Sensor; Polar, Kempele, Finland).

Data collection

Measurement of height was done using a stadiometer (SECA, Chino, CA, USA). Body weight was measured using a digital scale (Omron HN-289, Kyoto, Japan). BMI is calculated as weight in kilograms divided by height squared in meters. VO_{2max} was measured using the Astrand 6-min cycle test method using an ergocycle tool (Monark 828 E, Version 1010; Monark, Vansbro, Sweden). Measurement of RHR used a Pulse Oximeter (PO 30 Pulse Oximeter, Beurer,

Germany). Blood pressure was measured using an Omron HEM-7130 L automated device at the nondominant arm 3 times consecutively with a 1-2 min interval between two measurements while participants were in a seated position.

Blood collection and blood analysis

Blood sampling was performed in a cubital vein, and about 4 ml were taken after a 12 h overnight fasting (Daskalopoulou *et al.*, 2014; Elizondo-Montemayor *et al.*, 2017). Sampling was done 30 min before and 10 min after exercise. Blood was centrifuged for 15 min at 3,000 rpm. Serum was separated and stored at -80 °C for analysis of TNF- α and IL-6 levels during the next day (Daskalopoulou *et al.*, 2014; Tsuchiya *et al.*, 2014, 2015). The TNF- α level was measured using an ELISA kit (E-EL-H0109; Human TNF- α ELISA Kit; Elabscience, Houston, TX, USA) with kit sensitivity of 4.69 pg/ml and detection range 7.81-500 pg/ml. The IL-6 level was measured using an ELISA kit (E-EL-H0102; Human IL-6 ELISA Kit; Elabscience) with a kit sensitivity of 4.69 pg/ml and a detection range of 7.81-500 pg/ml. Measurement of fasting blood glucose (FBG) was done with an Accu-Chek Performa (Roche Diagnostics, Mannheim, Germany) with a concentration unit of mg/dl, while measurement of Hb was done using Mission Hb Test Strips, (ACON laboratories, Inc., San Diego, CA, USA) with a concentration unit of g/dl.

Statistical analysis

Statistical analysis using SPSS software version 21 (Chicago, IL, USA). The normality test used the Shapiro-Wilk test, while the homogeneity test used the Levene test. Difference

test used the paired sample t-test, one-way repeated measures ANOVA, followed by Tukey's Honestly Significant Difference (HSD) post hoc test. All data are shown with mean \pm standard deviation (SD). All statistical analyses used a significant level set at $P \leq 0.05$.

3. Results

The basic profiles of the samples, including age, height, weight, body mass index, haemoglobin, fasting blood glucose, systolic blood pressure, diastolic blood pressure, resting heart rate, maximal oxygen volume, oxygen saturation, are displayed in Table 1. Table 1 shows that the average data of research subjects including age, height, weight, body mass index, haemoglobin, fasting blood glucose, systolic blood pressure, diastolic blood pressure, resting heart rate, maximal oxygen volume, did not show significant differences in each group ($P \geq 0.05$).

The analysis of the paired sample t-test on CONG show that there was no significant difference in the mean TNF- α levels between pre-exercise vs post-exercise ($P > 0.05$) (Figure 1). Likewise, this was found for MIEG ($P > 0.05$). However, MCEG showed a significant difference in the mean TNF- α levels between pre-exercise vs post-exercise ($P \leq 0.05$) (Figure 1). The analysis of the IL-6 levels between pre-exercise vs post-exercise in each group is shown in Figure 2. The analysis of the paired sample t-test showed that there was no significant difference in the mean levels of IL-6 on CONG between pre-exercise vs post-exercise ($P > 0.05$) (Figure 2). However, MIEG and MCEG both showed a significant difference in the mean IL-6 levels between pre-exercise vs post-exercise ($P \leq 0.01$) (Figure 2).

Table 1. Characteristics of study subjects.^{1,2}

Parameter	Group			P-value
	CONG (n=8)	MCEG (n=8)	MIEG (n=8)	
Age (yrs)	21.12 \pm 1.46	21.37 \pm 1.41	21.25 \pm 1.75	0.949
Height (m)	1.58 \pm 0.04	1.57 \pm 0.05	1.59 \pm 0.06	0.851
Weight (kg)	73.87 \pm 6.84	71.94 \pm 7.54	72.64 \pm 8.36	0.876
BMI (kg/m ²)	29.55 \pm 1.76	28.94 \pm 1.45	28.82 \pm 1.76	0.650
Hb (g/dl)	15.76 \pm 1.88	15.02 \pm 0.97	15.07 \pm 1.01	0.489
FBG (mg/dl)	91.00 \pm 6.69	89.62 \pm 7.56	89.37 \pm 5.71	0.873
SBP (mmHg)	114.75 \pm 4.56	113.12 \pm 3.23	114.62 \pm 4.27	0.677
DBP (mmHg)	75.75 \pm 4.27	75.62 \pm 4.31	75.87 \pm 4.02	0.993
RHR (bpm)	73.12 \pm 6.79	72.12 \pm 4.85	72.62 \pm 5.21	0.940
VO _{2max} (ml/kg/min)	27.21 \pm 2.12	28.53 \pm 3.13	26.19 \pm 0.97	0.139
SpO ₂ (%)	97.62 \pm 1.51	97.50 \pm 1.41	97.25 \pm 1.28	0.863

¹ BMI = body mass index; Hb = haemoglobin; FBG = fasting blood glucose; SBP = systolic blood pressure; DBP = diastolic blood pressure; RHR = resting heart rate; VO_{2max} = maximum oxygen volume; SpO₂ = oxygen saturation; CONG = control group; MCEG = moderate-intensity continuous exercise group; MIEG = moderate-intensity interval exercise group.

² Data are presented as mean \pm standard deviation. P-value were obtained using one way-ANOVA.

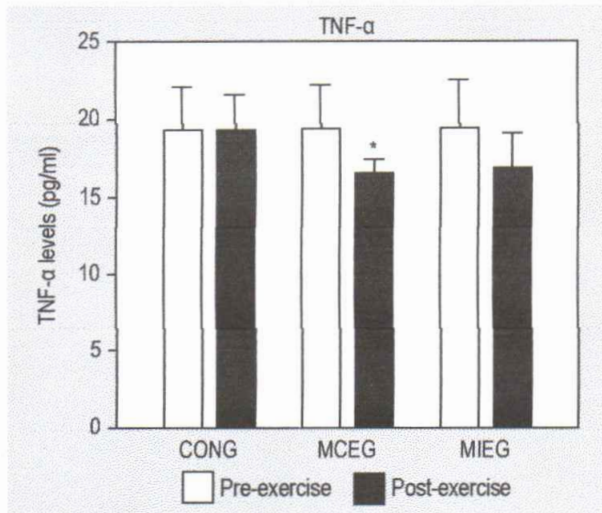


Figure 1. Tumour necrosis factor (TNF)-α levels pre-exercise versus post-exercise. CONG = control group; MCEG = moderate-intensity continuous exercise group; MIEG = moderate-intensity interval exercise group. Data are presented as mean ± standard deviation. P-value were obtained using paired samples t-test to compare post-exercise vs pre-exercise. * $P \leq 0.05$.

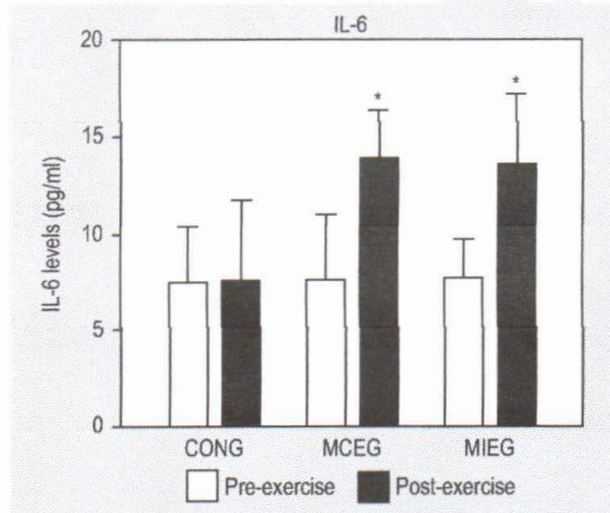


Figure 2. Interleukin 6 (IL-6) levels pre-exercise versus post-exercise. CONG = control group; MCEG = moderate-intensity continuous exercise group; MIEG = moderate-intensity interval exercise group. Data are presented as mean ± standard deviation. P-value were obtained using paired samples t-test to compare post-exercise vs pre-exercise. * $P \leq 0.05$.

Table 2. Tumour necrosis factor (TNF)-α and interleukin (IL)-6 levels in each group based on the blood collection time.¹

Parameter	Time	Group			P-value
		CONG (n=8)	MCEG (n=8)	MIEG (n=8)	
TNF-α (pg/ml)	pre-exercise	19.35±2.73	19.42±2.79	19.46±3.08	0.997
	post-exercise	19.36±2.23	16.63±0.82*	16.96±2.11*	0.014
IL-6 (pg/ml)	pre-exercise	7.56±2.88	7.68±3.41	7.78±1.99	0.988
	post-exercise	7.66±4.12	13.97±2.38*	13.66±3.55*	0.002

¹ CONG = control group; MCEG = moderate-intensity continuous exercise group; MIEG = moderate-intensity interval exercise group.

² Data are presented as mean ± standard deviation. One way-ANOVA, followed by Tukey's HSD post hoc test, was used to compare the differences among groups. * significant vs control group (CONG) ($P \leq 0.05$).

The analysis of TNF-α and IL-6 levels in each group based on the blood collection time is shown in Table 2.

The results of the one-way repeated measures ANOVA test showed no significant difference in the mean TNF-α levels of each group when measured in pre-exercise blood ($P > 0.05$), while the post-exercise showed a significant difference in the mean TNF-α levels ($P \leq 0.05$) (Table 2). The results of the Tukey's HSD post hoc test showed significant difference in the mean post-exercise TNF-α levels between MCEG and CONG ($P = 0.019$), MIEG and CONG ($P = 0.041$), while MCEG and MIEG did not show a significant difference ($P > 0.05$). The results of the one-way repeated measures ANOVA showed no significant difference in the mean levels of IL-6 of each group when

measured in pre-exercise blood ($P \geq 0.05$), whereas in the post-exercise there was a significant difference in the mean levels of IL-6 ($P \leq 0.05$). The results of the Tukey's HSD post hoc test showed that there was a significant difference in the mean post-exercise IL-6 levels between MCEG and CONG ($P = 0.004$), MIEG and CONG ($P = 0.006$), while MCEG and MIEG did not show a significant difference ($P > 0.05$).

4. Discussion

This study aimed to analyse the response of interval and continuous exercise to inflammatory markers in obese women. Based on the one-way repeated measures ANOVA test, the characteristics of the research subjects showed no significant difference ($P > 0.05$). These prove that the

subject is in the same physical condition before doing the exercise. The main finding in this study was an increase in IL-6 levels and a decrease in TNF- α levels in the interval and continuous groups 10 min post-exercise. During exercise, there is an increase in muscle contraction that causes muscle fibre damage and low glycogen, thereby triggering a response from skeletal muscles to repair muscle fibres (myogenesis) and replenish muscle glycogen by secreting IL-6 cytokines. Muñoz-Cánoves *et al.* (2013) stated that IL-6 signalling has been associated with stimulation of muscle hypertrophy and myogenesis through regulation of the proliferative capacity of muscle stem cells. Santos *et al.* (2019) in his research using obese adolescent subjects who were given an exercise intervention for 30 min, stated that there was a significant increase in IL-6 levels ($P \leq 0.05$). Similar results with this study in relation to TNF- α parameters have been carried out by Mauer *et al.* (2014) who demonstrated a low-grade inflammatory effect by administering a low dose of *Escherichia coli* endotoxin (0.06 ng/kg) to healthy volunteers, who were randomised to rest or exercise prior to infusion. The results show that in resting individuals, endotoxin has induced a 2- to 3-fold increase in circulating TNF- α . On the other hand, in participants who did 3 h of cycling the ergometer showed that exercise inhibited TNF- α production. An increase in IL-6 and a decrease in TNF- α during exercise certainly has a special relationship, because in addition to trigger glycogenolysis and lipolysis processes as energy reserves (Benatti and Pedersen, 2015), IL-6 secreted by muscles during exercise has an anti-inflammatory effect (Muñoz-Cánoves *et al.*, 2013) by stimulating IL-10 in circulation, interleukin 1 receptor antagonist (IL1RA), and the level of tumour necrosis factor-soluble receptor (sTNFr) which causing decrease in pro-inflammatory factors (Brandt and Pedersen, 2010; Cabral-Santos *et al.*, 2015; Lira *et al.*, 2015) like TNF- α (Metsios *et al.*, 2020). In addition, exercise is also believed to stimulate a phenotypic switch from M1-type macrophages that produce TNF, IL-6, and nitric oxide to M2-type macrophages, which release arginase and anti-inflammatory cytokines (Kawanishi *et al.*, 2010; Oliveira *et al.*, 2013). Kawanishi *et al.* (2010) stated that after exercise it will result in a decrease in tissue expression of intercellular adhesion molecule-1 (ICAM-1): which is involved in the adhesion of inflammatory cells to the endothelium and conveys the interaction of T cells with target cells, thereby inhibiting the migration of pro-inflammatory M1-type macrophages in adipose tissue.

Based on the analysis, no significant difference was found in the levels of IL-6 and TNF- α between the interval group and the continuous group, although when viewed from the mean levels of IL-6 and TNF- α in the continuous exercise group, it was shown to be more effective in increasing IL-6 and reduce TNF- α levels. However, when compared to the control group, IL-6 levels in the interval and continuous groups were higher and TNF- α levels were lower after

exercise. This is similar to the research conducted by Newlin *et al.* (2012) which reported that moderate-intensity continuous exercise (65% VO_{2max}) significantly increased IL-6 levels. Results related to TNF- α parameters in this study are similar to the research conducted by Hoseini *et al.* (2018) in which male smokers and non-smokers were given a continuous aerobic exercise intervention for 40 min with an intensity of 70% HR_{max} ; the result was that TNF- α levels decreased. In addition, Lira *et al.* (2017) have also proven in their research with active male subjects, performing aerobic exercise using a treadmill continuously with a distance of 5 km and a speed of 70% of the maximum aerobic capacity, that IL-6 levels increased 60 min post-exercise and TNF- α levels decreased post-exercise. The high increase in IL-6 and decrease in TNF- α in continuous exercise correlated with a higher rate of muscle contraction in continuous exercise compared to interval training. When muscle contraction increases, it causes muscle fibre damage and low glycogen, thereby triggering a response from skeletal muscle to repair muscle fibres (myogenesis) (Muñoz-Cánoves *et al.*, 2013) and replenish muscle glycogen by secreting the cytokine IL-6 (Benatti and Pedersen, 2015). High levels of IL-6 cause TNF- α decrease by stimulating an increase in circulating IL-10, IL1RA, and sTNFr levels that specifically reduce pro-inflammatory factors (Brandt and Pedersen, 2010; Cabral-Santos *et al.*, 2015; Lira *et al.*, 2015).

IL-6 is the myokine prototype, although most rheumatologists may view IL-6 as a proinflammatory cytokine. There is evidence that IL-6 released from muscle during exercise has an anti-inflammatory function (Muñoz-Cánoves *et al.*, 2013; Pal *et al.*, 2014). This is because the signalling pathways for IL-6 secreted by myocytes with macrophages are very different (Pedersen and Febbraio, 2008). Unlike IL-6 signalling in macrophages, which depend on activation of the nuclear factor- κ B (NF- κ B) signalling pathway, intramuscular IL-6 expression is regulated by a network signalling cascade involving crosstalk between Ca^{2+} and nuclear factor of activated T-cells (Muñoz-Cánoves *et al.*, 2013). Therefore, when IL-6 is activated by macrophages, a proinflammatory response will be created, while the increase in IL-6 secreted by myocytes does not depend on the TNF- α response or by NF- κ B activation (Muñoz-Cánoves *et al.*, 2013) so it is anti-inflammatory because it can stimulate an increase in anti-inflammatory cytokines, i.e. IL1RA and IL-10, so that it can decrease pro-inflammatory cytokines (Pedersen, 2009). In addition, during inflammatory conditions IL-6 has also been shown to limit the expression of genes encoding inflammatory cytokines (e.g. TNF- α , IL-1 β , nitric oxide synthase 2) and activation of terminal c-Jun N kinase which then enhances macrophage responsiveness to IL-4, thereby greatly supporting the idea that IL-6 can reduce inflammation levels (Mauer *et al.*, 2014). This is very different from the signalling pathway of TNF- α , which is only mediated by macrophage activation so that the high increase in TNF- α

only occurs in chronic inflammation (Vijayaraghava and Doreswamy, 2017).

Limitations to the current study include (1) the small sample size, (2) determination of obesity using only BMI, and (3) subjects only performing acute exercise. Firstly, in this study, we only used a small sample size with the total number of subjects 24 obese women. Therefore, a future study should include a larger number of women obese subjects. Secondly, the use of BMI to determine obesity has low accuracy because it does not describe human composition, such as free fat mass, fat mass, and fat distribution (Akpinar *et al.*, 2007). Therefore, further research is recommended to measure free fat mass, fat mass, percentage body fat, and muscle mass using dual-energy absorptiometry (DEXA) as a determinant of obesity. Thirdly, this study only studied acute intervention (exercise), so that further research is needed to determine the effect of the chronic intervention (training) on obese women. The results are expected to be useful for stabilising optimal exercise programmes in obesity management in the future.

5. Conclusions

Based on the study results, it can be concluded that single sessions of moderate-intensity continuous and interval exercise performed for 30-35 min significantly reduced pro-inflammatory and increased anti-inflammatory cytokines.

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Conflict of interest

The authors declare that they have no competing interests.

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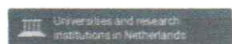
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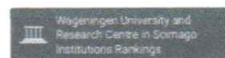
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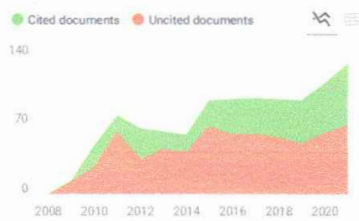
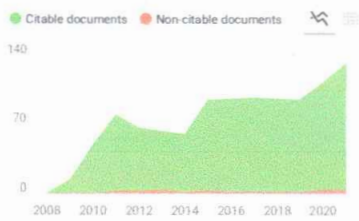
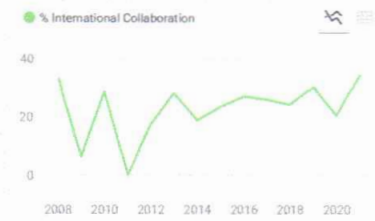
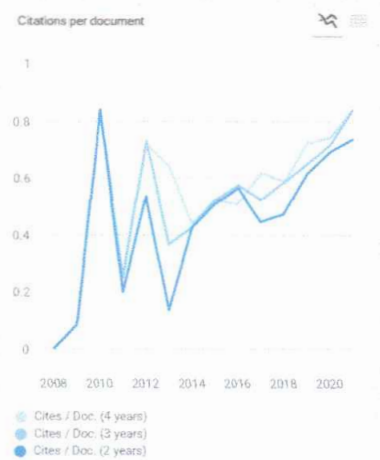
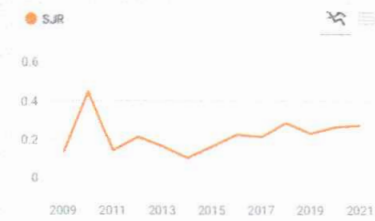
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