JPJO 8 (2) (2023)) 148-162

Jurnal Pendidikan Jasmani dan Olahraga

Available online at:
https://ejournal.upi.edu/index.php/penjas/article/view/60606
DOI: https://doi.org/10.17509/jpjo.v8i2.60606

Exercise in Polluted Spaces: Inflammatory Differentiation and Lung Mitochondrial Biogenesis

Lucky Angkawidjaja Roring[1*], Amung Ma'mun[1], Hamidie Ronald Daniel Ray[1], Beta Paramita[2]

[1]Department of Sports Education, Postgraduate Program, Universitas Pendidikan Indonesia, Indonesia
[2]Architecture Undergraduate Program, Universitas Pendidikan Indonesia, Indonesia

Article Info

Article History :
Received July 2023
Revised August 2023
Accepted August 2023
Available online September 2023

Keywords :
Exercise, Inflammation, Mitochondrial Biogenesis

Abstract

In-

Indonesia is ranked as the most highly polluted country in Southeast Asia and the 26th on the list of the most polluted countries worldwide. Exercising in polluted spaces can have complex effects on inflammation and mitochondrial biogenesis in the lungs. The interaction between physical activity and pollution exposure may affect lung health. This study aimed to analyse the different effects of exercise in polluted open spaces and antioxidant administration on lung inflammation and mitochondrial biogenesis. The research approach used was a quantitative approach with a comparative descriptive design using random assignment technique. The research sample used male white rats (Rattus Norvegicus) Wistar strain aged 8-10 weeks with a body weight of 200-300 g obtained from Biofarma Animal Breeding Facility. The sampling technique used in this study was random assignment technique. In this study, the number of members of the treatment group was 5, so the total research sample amounted to 5 x 9, namely 45 samples. The results showed that there was a differentiation in the level of inflammation represented by the level of IL-6 and NF-κB and lung mitochondrial biogenesis level represented by PGC-1α, TOM20, and COX IV with moringa administration in physical activities, as indicated by the comparison of the mean values of the inflammatory process and lung mitochondrial biogenesis in the nine treatment groups. The implication of this research is that it is necessary to consider appropriate sport strategies or guidelines for individuals who exercise in a polluted environment to reduce the negative impact on the respiratory system and overall lung health.

✉ Correspondence Address : Jl. Dr Setiabudhi No 229, Kota Bandung, Jawa Barat 40154
E-mail : luckyroring@upi.edu

INTRODUCTION

Indonesia is ranked as the most highly polluted country in Southeast Asia and 26th on the list of most polluted countries worldwide. People living in areas with high pollution levels can face a number of risks and threats to their health and quality of life. The main risks that can occur are: (1) respiratory problems such as coughing, shortness of breath, and throat irritation; (2) increased risk of developing chronic lung diseases, such as COPD, and cardiovascular diseases, such as coronary heart disease; (3) increased risk of premature death from various pollution-related diseases, including respiratory and cardiovascular diseases; and (4) the resident work productivity and quality of life (Sudaryanto et al., 2022; Arsyad & Priyana, 2023). Air pollution is a human rights issue. Air pollution on the current scale clearly violates the right of life and health. Clean air and clean water are essential for human health and well -being. Everyone has the right to breathe clean air (Boyd et al., 2019).

Pulmonary inflammation is the body response to injury or infection that causes inflammation and stimulates the immune system to fight against pathogens or harmful substances. Meanwhile, pulmonary mitochondrial biogenesis is the process of formation and multiplication of mitochondria in cells to maintain cellular energy balance and lung cell function. There is a link between intensive physical activity and an increased risk of lung inflammation (Calvano, 2005). High exposure to air pollution, including particulate matter and harmful chemicals, such as ozone (Rüsch et al., 2005), nitrogen dioxide, and other pollutants, can cause inflammation of the airways (Elinav, 2013).

Exercise in polluted ambient can increase exposure to harmful substances, and in the short term, can cause symptoms, such as coughing (Strobel, 2011), shortness of breath, and increased sputum production (Smith et al., 2023). However, in the long term, repeated lung inflammation due to exercise in polluted ambient may contribute to the development of chronic lung diseases (Nomoto et al., 2009). For example, individuals who have a habit of regular physical exercise have low cytokines, low levels of inflammation-inducing skeletal muscle proteins (Elias, 2008), low adipokine production, and low serum CRP levels (Albert, 2001). Therefore, although strenuous exercise is potentially stressful, and can decrease immune function and susceptibility to infection, but it does not impair an individual overall immunity. The process of increasing or decreasing immune activation as well as inflammation is one of the normal mechanisms. The mechanism of increasing or decreasing immune and inflammatory activity includes long- term health benefits that can be obtained by individuals by doing moderate and regular physical exercise (Mileva & Zaidell, 2022). Individuals who regularly perform physical exercise can reduce the level of biomarkers in systemic inflammation (Hagger & Smith, 2018).

Therefore, it is important to remember that exercise in polluted spaces can have a complex impact on inflammation and mitochondrial biogenesis in the lungs (Heinonen, 2015; Stehling, 2014). Inflammation is the body natural response to exposure to foreign substances or tissue damage, while mitochondrial biogenesis is the process of forming new mitochondria in cells (Gureev, 2019). The interaction between physical activity and pollution exposure can affect lung health. Under polluted conditions, lung health can be affected by a combination of inflammation and mitochondrial biogenesis. Exposure to air pollution can lead to excessive lung inflammation, while exercise in polluted conditions can contribute to higher ROS (Reactive Oxygen Species) production and oxidative stress. Accumulated inflammation and oxidative stress can lead to cell and mitochondrial damage and disrupt the balance between inflammation and mitochondrial biogenesis (Oliveira, 2016; Pfanner, 2019).

In recent years, research on the impact of exercise activities in polluted spaces on inflammatory differentiation and mitochondrial lung biogenesis has become an interesting research focus. Several studies have tried to understand the mechanism of interaction between exercise, air pollution, and lung health. The study by (Zhang, 2007) showed that regular exercise could reduce lung inflammation levels and increased mitochondrial biogenesis, indicating a possible positive effect of exercise on lung conditions during pollution exposure. The study of (Freeman, 2013) showed that aerobic exercise could reduce lung inflammation and reduce oxidative stress, which might contribute to better lung health under polluted conditions. The study of Liu et al. (2021) showed that regular exercise could modulate the expression of inflammatory and mitochondrial genes in white blood cells, suggesting that exercise could have

beneficial effects on lung health under polluted conditions. However, it is important to remember that research on this topic is still evolving and there is no clear consensus on all aspects of it, including long-term effects, more in-depth molecular mechanisms, and intervention and treatment of studies.

Therefore, this study was conducted with a more specific focus, namely on the differentiation of lung inflammation and mitochondrial biogenesis in exercise activities in polluted spaces, conducted in different groups with various interventions and specific treatments. This study aimed to analyze the differentiation of the effects of exercise in polluted outdoor spaces and the administration of antioxidants on lung inflammation and mitochondrial biogenesis. The implication of this study is that it is necessary to consider appropriate exercise strategies or guidelines for individuals who exercise in polluted environments to reduce the negative impact on the respiratory system and overall lung health .

METHODS

The approach used in this study was the quantitative approach (Cresswell & Clark, 2011) aimed at producing data in the form of numbers while describing the results of empirical tests on the effect of exercise in polluted open spaces and antioxidant administration on lung inflammation and mitochondrial biogenesis. The research used descriptive comparative design using random assignment technique (Cresswell & Clark, 2011) .

Participants

The study population were appropriate test animals that met the criteria. The test animals were male white rats (Rattus norvegicus) Wistar strain aged 8-10 weeks with a weight of 200-300 g obtained from Biofarma Animal Breeding Facility. The reason Wistar strain rats were used in this study was because of their similarity to humans in many biological and physiological aspects. Therefore, the results of studies using Wistar strain rats can provide relevant insights into human health and disease.

There were three sample criteria in this study, namely inclusion criteria, exclusion criteria, and test dropout criteria. Inclusion criteria included male Wistar rats (Rattus norvegicus), aged 8-10 weeks, weight 200-225 grams, and had normal behavior and activity. Exclusion criteria included rats that had visible skin abnormalities (wounds, ulcers), rats whose hair looked dull and falling out, and there were injuries during exercise treatment. Test dropout criteria included sick rats with signs of weight loss > 10% after the adaptation period in the laboratory and rats died during the adaptation period.

Sampling Procedures

The sampling technique used in this study was the random assignment technique. The random assignment sampling technique is the process of randomly assigning research sample groups to different treatment conditions (Kirschner et al., 2019). The research sample was selected based on Federer's formula, namely (t-1)(n-1) ≥15). t = number of groups, n = number of subjects per group. Based on Federer's formula, the number of group members ≥ 2,875 was obtained. In this study, the number of members of the treatment group was 5, so the total research sample amounted to 5 x 9, namely 45 samples.

Materials and Apparatus

The equipment used in this study included the following, (1) rat treadmill, made according to physical exercise standards, (2) 9 in 1 CO2 TVOC Formaldehyde Humidity Meter Air quality monitor, a tool for measuring air quality with particulate matter parameters and PM2.5, PM10, and AQI, (3) CO meter, a tool for measuring carbon monoxide levels, (4) sample preparation equipment, (5) sonde, a tool for giving doses of antioxidants, (6) gel making equipment, and (7) electrophoresis equipment. The materials used in this study included the following, (1) vitamin C tablets 50 mg, (2) Moringa oleifera leaf extract, (3) carboxymethylcellulose (CMC) powder, (4) materials for making lung tissue research samples, and (5) gel making materials used in electrophoresis.

Procedures

Broadly speaking, the experimental procedure was divided into three stages, namely the initial/preparation stage, the core/implementation stage, and the final/closing stage. In the initial/preparation stage, the activities carried out were maintaining and preparing test animals. In the core/implementation stage, the activities carried out were the treatments of test animals, by determining and giving treatments to test animals into

nine groups. In the final/closing stage, the activity carried out was collecting data on protein expression of IL-6, NF-κB, PGC-1α, TOM20, and COX IV. The following steps were instrument preparation of lung tissue samples, gel making, electrophoresis, electro-transfer, and protein detection

Design or Data Analysis

A comparative design was used. The criteria for comparison included that inflammation should have a large mean and small coefficient of variation, while mitochondrial biogenesis should have a large mean and small coefficient of variation.

RESULT

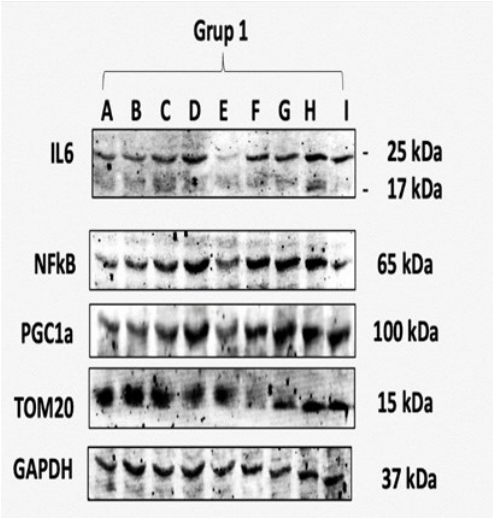
The differentiation of marker components used to show the occurrence of lung inflammation in this study was limited to IL-6 (cytokine) and NF-κB (transcription factor) which have important roles in inflammation and immune response processes. The biomarkers of mitochondrial biogenesis were represented by PGC-1α, TOM20, and COX IV.

The sample group was sorted into nine groups, namely (1) no exercise group, (2) no exercise group with antioxidant Moringa, (3) no exercise group with pollutants, (4) no exercise group with antioxidant vitamin C, (5) no exercise group with pollutants and antioxidant Moringa, (6) exercise group, (7) exercise group with pollutants, (8) exercise group with antioxidant Moringa, and (9) exercise group with pollutants and antioxidant Moringa.

Data obtained from laboratory tests on 45 samples sorted into five sampling unit groups and nine treatment groups are presented in Figure 1.

The Western blot results of all sample groups are outlined in Table 1. Before presenting the research results and discussion, the research data and sample statistical measures were firstly presented as reference materials in data analysis as well as the formulation of results and discussion of research results.

The average conditions of the biomarkers of the inflammatory process in the lungs, namely IL-6 and NF - κB, of the samples are presented in Figure 2. The figure shows a comparison of the average values of the lung inflammation process of the samples in the nine treatment groups. The IL-6 marker is shown in the blue color image while NF-κB is shown in the orange color image.



Dscripsion :

A: Without exercise
B: No exercise + Moringa
C: No exercise + Pollution
D: No exercise + Vitamin C
E: No exercise + Moringa + Pollution
F: Exercise
G: Exercise + Pollution
H: Exercise + Moringa
I: Exercise + Moringa + Pollution

Figure 1. Western Blot of Samples

Meanwhile, the average values of mitochondrial biogenesis function represented by PGC-1α, TOM20, and COX IV are presented in Figure 3.

The figure shows a comparison of the average value of mitochondrial function biogenesis of samples in nine treatment groups. The PGC-1α biomarker is shown in blue, the TOM20 biomarker is shown in orange, and the COX IV biomarker is shown in gray. To interpret the effect size, Cohen's (1988) criteria were used.

The results of descriptive statistics calculation of the mean and standard deviation of inflammation and mitochondrial lung biogenesis can be seen in Table 3.

Based on the average results of the IL-6 component, the largest was group A and the smallest was group E. Five groups showed above average IL-6, namely groups A, C, G, F, B, while four groups showed below average IL-6, namely groups D, H, I, E. In general, the research findings showed that the IL-6 component tended to be high.

Table 1. Western blot data for IL-6, NF-κB, PGC-1α, TOM20, and COX IV of 45 subjects in nine sample groups

Group	IL-6	NF-κB	PGC-1α	TOM20	COX IV
A3	1.00640445	0.82278550	0.84129545	1.06552023	1.07476783
A10	0.98829497	0.73459010	0.62752512	0.79568663	0.78979503
A14	0.81942087	0.70347808	0.83279872	0.63487111	0.69892026
A15	0.88886270	0.85821626	0.79854118	0.75008688	0.93472052
A30	0.35325299	0.57821993	0.62679007	0.99372051	0.99329309
Average	**0.81124720**	**0.73945797**	**0.74539011**	**0.84797707**	**0.89829935**
B11	0.93787252	0.84675464	0.81428741	1.07593004	0.93988663
B21	0.60968790	0.85095326	0.92175036	0.84053100	0.85198620
B23	0.80322762	0.72680373	0.70656638	0.64324499	0.67332614
B39	0.74379434	0.59634851	0.53213476	0.80623932	0.96033798
B42	0.48090000	0.74983368	0.72015887	0.98354719	0.99336311
Average	**0.71509648**	**0.75413876**	**0.73897956**	**0.86989851**	**0.88378001**
C1	1.01713159	0.95114781	0.85052198	1.00906107	0.85707243
C19	0.86273203	0.85738267	0.83509771	0.88212946	0.89599682
C24	0.76179676	0.73120859	0.41667593	0.49007801	0.49265582
C26	0.76161008	0.51315518	0.53196247	0.95268188	0.99154764
C35	0.62850924	0.89754691	0.76715098	0.46721930	0.60272319
Average	**0.80635594**	**0.79008823**	**0.68028182**	**0.76023394**	**0.76799918**
D4	1.10216406	1.08647109	1.03079255	0.81834407	0.63116958
D7	0.32132428	0.44144724	0.69055922	0.78472399	0.75777348
D22	0.77123999	0.48174372	0.68114881	0.77617589	0.79696929
D28	0.91666768	0.79582993	0.73718113	0.45112329	0.61721010
D33	0.34255812	0.62016838	0.52212199	0.77255840	0.84091621
Average	**0.69079083**	**0.68513207**	**0.73236074**	**0.72058513**	**0.72880773**
E8	0.50310305	0.91288015	0.89848240	0.90777159	0.63990162
E12	0.20491289	0.32639438	0.76445508	0.65926630	0.55764667
E13	0.69100690	0.48221467	0.65005274	0.73854492	0.72857010
E17	0.87303929	0.73958767	0.57863254	0.65380745	0.80859904
E18	0.64520679	0.63321518	0.52938894	0.97809015	0.97858728
Average	**0.58345378**	**0.61885841**	**0.68420234**	**0.78749608**	**0.74266094**
F5	1.04071386	1.09488773	1.03304592	0.54043177	0.64849274
F9	0.68897324	0.65933771	0.69821910	0.75660825	0.71765777
F25	0.61356611	0.41294785	0.37959757	0.87664350	0.86591685
F31	0.61603994	0.50295407	0.49365602	0.92212483	0.99597650
F32	0.66364399	0.69919369	0.59305714	0.80765878	0.77397758
Average	**0.72458743**	**0.67386421**	**0.63951515**	**0.78069343**	**0.80040429**
G16	1.08858220	1.15781628	1.11235719	0.79621441	0.80328238
G20	0.64910348	0.70336773	0.90531504	0.77341881	0.76390664
G27	0.66284006	0.58543136	0.80772885	0.84800446	0.88359835
G34	0.77672047	0.73975573	0.63367952	0.98943807	0.97392186
G43	0.52808029	0.42634910	0.48129517	0.91455061	0.95599264
Average	**0.74106530**	**0.72254404**	**0.78807515**	**0.86432527**	**0.87614037**
H29	1.08159686	1.04757100	0.99765582	0.90945824	0.97597656
H36	0.36602236	0.51758324	0.97129775	0.64499941	0.58600950
H38	0.54247610	0.59798509	0.82856376	0.96983835	0.96727017
H41	0.46419208	0.40150705	0.50481224	0.98809224	1.02256859
H40	0.82903858	0.97012738	0.78087079	1.01151964	0.94587419

Group	IL-6	NF-κB	PGC-1α	TOM20	COX IV
Average	**0.65666520**	**0.70695475**	**0.81664007**	**0.90478158**	**0.89953980**
I2	0.97591148	0.76517958	0.99096626	0.86818528	0.93912404
I6	0.47086220	0.43832881	0.96221362	0.43755329	0.38531477
I44	0.68246157	0.79754922	0.83306016	0.73468979	0.72148355
I45	0.71222977	0.51726703	0.56771317	0.81860727	0.99180485
I37	0.34445775	0.44859947	0.40058122	0.72302039	0.77514234
Average	**0.63718455**	**0.59338482**	**0.75090689**	**0.71641121**	**0.76257391**

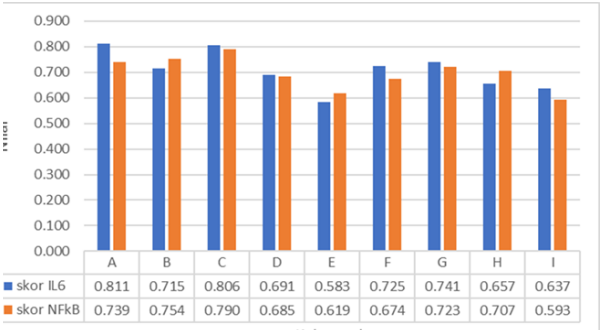


Figure 2. Average Lung Inflammatory Process of Samples in Nine Treatment Groups

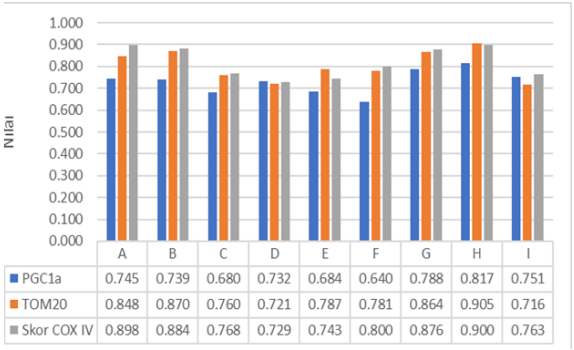


Figure 3. Average Mitochondrial Biogenesis Function of Samples in Nine Treatment Groups

Table 2. Suggested Effect Size Magnitude Chart

Effect Size Calculation	Test Statistics	Small Effect	Medium Effect	Large Effect
Phi of Cramer's Phi	Chi Squared	0.1	0.3	0.5
Cohen's d		0.2	0.5	0.8
Eta Squared	ANOVA	0.01	0.06	0.14
R	Correlation	0.1	0.3	0.5
r^2	Correlation and t-test (independent)	0.01	0.09	0.25

Table 3. Mean and Standard Deviation of IL-6, NF-κB, PGC-1α, TOM20, and COX IV in Nine Sample Groups Sorted from Largest to Smallest

Component	Group	Mean	Std. Deviation	N
IL-6	A: Without exercise	0.8112472	0.26705895	5
	C: No exercise + Pollution	0.80635594	0.14424094	5
	G: Exercise + Pollution	0.7410653	0.21329444	5
	F: Exercise	0.72458743	0.17960142	5
	B: No exercise + Moringa	0.71509648	0.17619505	5
	D: No exercise + Vitamin C	0.69079083	0.34802726	5
	H: Exercise + Moringa	0.6566652	0.29361446	5
	I: Exercise + Moringa + Pollution	0.63718455	0.24278068	5
	E: No exercise + Moringa + Pollution	0.58345378	0.24949465	5
	Total	.7073829667	.23079967160	45
NF-κB	C: No exercise + Pollution	0.79008823	0.17476758	5
	B: No exercise + Moringa	0.75413876	0.10442012	5
	A: Without exercise	0.73945797	0.1099579	5
	G: Exercise + Pollution	0.72254404	0.27233124	5
	H: Exercise + Moringa	0.70695475	0.28561929	5
	D: No exercise + Vitamin C	0.68513207	0.26376904	5

Component	Group	Mean	Std. Deviation	N
	F: Exercise	0.67386421	0.26239333	5
	E: No exercise + Moringa + Pollution	0.61885841	0.22662589	5
	I: Exercise + Moringa + Pollution	0.59338482	0.17463936	5
	Total	.6982692529	.20660650026	45
PGC-1α	H: Exercise + Moringa	0.81664007	0.19706846	5
	G: Exercise + Pollution	0.78807515	0.24353761	5
	I: Exercise + Moringa + Pollution	0.75090689	0.25754427	5
	A: Without exercise	0.74539011	0.10911148	5
	B: No exercise + Moringa	0.73897956	0.14427928	5
	D: No exercise + Vitamin C	0.73236074	0.18550953	5
	E: No exercise + Moringa + Pollution	0.68420234	0.148858	5
	C: No exercise + Pollution	0.68028181	0.19492474	5
	F: Exercise	0.63951515	0.24965126	5
	Total	.7307057578	.18698103524	45
TOM20	H: Exercise + Moringa	0.90478158	0.15006225	5
	B: No exercise + Moringa	0.86989851	0.16707167	5
	G: Exercise + Pollution	0.86432527	0.08852293	5
	A: Without exercise	0.84797707	0.17769152	5
	E: No exercise + Moringa + Pollution	0.78749608	0.14790541	5
	F: Exercise	0.78069343	0.1485279	5
	C: No exercise + Pollution	0.76023394	0.2610802	5
	D: No exercise + Vitamin C	0.72058513	0.15171671	5
	I: Exercise + Moringa + Pollution	0.7164112	0.1670471	5
	Total	.8058224680	.16503963106	45
COX IV	H: Exercise + Moringa	0.8995398	0.17749239	5
	A: Without exercise	0.89829935	0.15249315	5
	B: No exercise + Moringa	0.88378001	0.12876258	5
	G: Exercise + Pollution	0.87614037	0.09205694	5
	F: Exercise	0.80040429	0.13524317	5
	C: No exercise + Pollution	0.76799918	0.21060714	5
	I: Exercise + Moringa + Pollution	0.76257391	0.23868788	5
	E: No exercise + Moringa + Pollution	0.74266094	0.1620165	5
	D: No exercise + Vitamin C	0.72880773	0.10005069	5
	Total	.8178006207	.16126919488	45

The results of this study showed that in the absence of exercise, lung tissue inflammation could occur (Group A). Higher IL-6 levels also occurred in the exercise group with pollution (Group G) and the exercise group in open space without pollution (Group F). Low IL-6 levels were found in the group without exercise in polluted open space with Moringa administration (Group E) followed by the group with exercise in polluted open space with Moringa administration (Group I) and exercise in open space with Moringa administration (Group H).

Based on the average results of the NF-κB component, the largest was group C and the smallest was group I. Five groups showed above average IL-6, namely groups C, B, A, G, H, while four groups showed below average IL-6, namely groups D, F, E, I. The findings showed that the NF-κB component tended to be high.

The research findings on NF-κB levels are in line with the results of lung IL-6 level measurement, showing a tendency that the NF-κB component was high in the group without exercise accompanied by air pollution and tended to be low in the group with Moringa administration. It indicates that Moringa administration could reduce the level of inflammation in both polluted and non-polluted outdoor exercise groups.

Based on the average results of the PGC-1α component, the largest was group H and the smallest was group F. Six groups showed above average PGC-1α, namely groups H, G, I, A, B, D, while 3 groups showed below average PGC-1α, namely groups E, C, F. The findings showed that the IL-6 component tended to be high.

Table 4. Results of Coefficient of Variation on Inflammation and Lung Mitochondrial in Nine Groups

Component	Group	Average	Standard Deviation	Total	Coefficient of Variance
IL-6	D: No exercise + Vitamin C	0.690791	0.348027	5	50.38099
IL-6	H: Exercise + Moringa	0.656665	0.293614	5	44.71296
IL-6	E: No exercise + Moringa + Pollution	0.583454	0.249495	5	42.76168
NF-κB	H: Exercise + Moringa	0.706955	0.285619	5	40.40135
PGC-1α	F: Exercise	0.639515	0.249651	5	39.03758
NF-κB	F: Exercise	0.673864	0.262393	5	38.93861
NF-κB	D: No exercise + Vitamin C	0.685132	0.263769	5	38.49901
IL-6	I: Exercise + Moringa + Pollution	0.637185	0.242781	5	38.1021
NF-κB	G: Exercise + Pollution	0.722544	0.272331	5	37.69061
NF-κB	E: No exercise + Moringa + Pollution	0.618858	0.226626	5	36.61999
TOM20	C: No exercise + Pollution	0.760234	0.26108	5	34.34209
PGC-1α	I: Exercise + Moringa + Pollution	0.750907	0.257544	5	34.29776
IL-6	A: Without exercise	0.811247	0.267059	5	32.91955
COX IV	I: Exercise + Moringa + Pollution	0.762574	0.238688	5	31.30029
PGC-1α	G: Exercise + Pollution	0.788075	0.243538	5	30.90284
NF-κB	I: Exercise + Moringa + Pollution	0.593385	0.174639	5	29.43105
IL-6	G: Exercise + Pollution	0.741065	0.213294	5	28.78214
PGC-1α	C: No exercise + Pollution	0.680282	0.194925	5	28.65353
COX IV	C: No exercise + Pollution	0.767999	0.210607	5	27.42283
PGC-1α	D: No exercise + Vitamin C	0.732361	0.18551	5	25.33035
IL-6	F: Exercise	0.724587	0.179601	5	24.78671
IL-6	B: No exercise + Moringa	0.715096	0.176195	5	24.63934
PGC-1α	H: Exercise + Moringa	0.81664	0.197068	5	24.13162
TOM20	I: Exercise + Moringa + Pollution	0.716411	0.167047	5	23.31721
NF-κB	C: No exercise + Pollution	0.790088	0.174768	5	22.12001
COX IV	E: No exercise + Moringa + Pollution	0.742661	0.162017	5	21.81568
PGC-1α	E: No exercise + Moringa + Pollution	0.684202	0.148858	5	21.75643
TOM20	D: No exercise + Vitamin C	0.720585	0.151717	5	21.05465
TOM20	A: Without exercise	0.847977	0.177692	5	20.95476
COX IV	H: Exercise + Moringa	0.89954	0.177492	5	19.73147
PGC-1α	B: No exercise + Moringa	0.73898	0.144279	5	19.52412
TOM20	B: No exercise + Moringa	0.869899	0.167072	5	19.20588
TOM20	F: Exercise	0.780693	0.148528	5	19.02512
TOM20	E: No exercise + Moringa + Pollution	0.787496	0.147905	5	18.78173
IL-6	C: No exercise + Pollution	0.806356	0.144241	5	17.888
COX IV	A: Without exercise	0.898299	0.152493	5	16.97576
COX IV	F: Exercise	0.800404	0.135243	5	16.89686
TOM20	H: Exercise + Moringa	0.904782	0.150062	5	16.58547
NF-κB	A: Without exercise	0.739458	0.109958	5	14.87007
PGC-1α	A: Without exercise	0.74539	0.109111	5	14.63817
COX IV	B: No exercise + Moringa	0.88378	0.128763	5	14.56953
NF-κB	B: No exercise + Moringa	0.754139	0.10442	5	13.84627
COX IV	D: No exercise + Vitamin C	0.728808	0.100051	5	13.728
COX IV	G: Exercise + Pollution	0.87614	0.092057	5	10.5071
TOM20	G: Exercise + Pollution	0.864325	0.088523	5	10.24185

Based on the average results of the TOM20 component, the largest was group H and the smallest was group I. Four groups showed above average TOM20, namely groups H, B, G, A, while five groups showed below average TOM20, namely groups E, F, C, D, I. The research findings showed that the TOM20 component tended to be high.

Based on the average results of the COX IV component, the largest was group H and the smallest was group D. Four groups showed above average COX IV, namely groups H, A, B, G, while five groups showed below average COX IV, namely groups F, C, I, E, D. The research findings indicate that the COX IV component tended to be high.

The details to determine the coefficient of variation of each inflammatory component and lung mitochondrial biogenesis can be seen in Table 5.

flammation could be a sign or risk factor for various disease conditions. Inflammation that is controlled and within normal levels is important for maintaining a balanced and normal body. Administration of moringa had been shown to reduce inflammation represented by IL-6 level. It is important to note that inflammation is also the body normal response to injury or acute infection. In this context, inflammation is a natural protective mechanism that aids recovery. Therefore, it is important to understand that a low level of inflammation does not mean no inflammation at all, but rather refers to a normal and controlled level of inflammation.

Lower inflammation in subjects is considered better as it is associated with better health and reduced risk of chronic diseases. However, any assessment of inflammation should be done in a clinical context and consider other factors relevant to the condition of the

Table 5. Results of Coefficient of Variation of Lung Inflammation on IL-6 Component

Component	Group	Average	Standard Deviation	Total	Coefficient of Variance
IL-6	D: No exercise + Vitamin C	0.690791	0.348027	5	50.38099
IL-6	H: Exercise + Moringa	0.656665	0.293614	5	44.71296
IL-6	E: No exercise + Moringa + Pollution	0.583454	0.249495	5	42.76168
IL-6	I: Exercise + Moringa + Pollution	0.637185	0.242781	5	38.1021
IL-6	A: Without exercise	0.811247	0.267059	5	32.91955
IL-6	G: Exercise + Pollution	0.741065	0.213294	5	28.78214
IL-6	F: Exercise	0.724587	0.179601	5	24.78671
IL-6	B: No exercise + Moringa	0.715096	0.176195	5	24.63934
IL-6	C: No exercise + Pollution	0.806356	0.144241	5	17.888
	Total	0.707383			

Table 6. Results of the Coefficient of Variance of Lung Inflammation in the NF-κB Component

Component	Group	Average	Standard Deviation	Total	Coefficient of Variance
NF-κB	H: Exercise + Moringa	0.706955	0.285619	5	40.40135
NF-κB	F: Exercise	0.673864	0.262393	5	38.93861
NF-κB	D: No exercise + Vitamin C	0.685132	0.263769	5	38.49901
NF-κB	G: Exercise + Pollution	0.722544	0.272331	5	37.69061
NF-κB	E: No exercise + Moringa + Pollution	0.618858	0.226626	5	36.61999
NF-κB	I: Exercise + Moringa + Pollution	0.593385	0.174639	5	29.43105
NF-κB	C: No exercise + Pollution	0.790088	0.174768	5	22.12001
NF-κB	A: Without exercise	0.739458	0.109958	5	14.87007
NF-κB	B: No exercise + Moringa	0.754139	0.10442	5	13.84627
	Total	0.698269			

Groups E and I in Table 5 and Table 6 showed the consistency of moringa administration as evidenced by markers, alignment of IL-6 and NF-κB, and a decrease in lung inflammation. It showed that it provided a good inflammatory impact because excessive or chronic inflammation could be a sign or risk factor for various disease conditions. Inflammation that is controlled and within normal levels is important for maintaining a balanced and normal body. Administration of moringa had been shown to reduce inflammation represented by IL-6 level. It is important to note that inflammation is also the body normal response to injury or acute infection. In this context, inflammation is a natural protective mechanism that aids recovery. Therefore, it is important to understand that a low level of inflammation does not mean no inflammation at all, but rather refers to a normal and controlled level of inflammation.

Lower inflammation in subjects is considered better as it is associated with better health and reduced risk of chronic diseases. However, any assessment of inflammation should be done in a clinical context and consider other factors relevant to the condition of the subject being observed.

In Table 7, group H showed that the administration of moringa with exercise had a favorable impact on PGC-1α and stimulated mitochondrial formation. A large value of mitochondrial biogenesis is generally

considered favorable because it can improve energy efficiency, healthy metabolism, oxidative protection, and physical adaptation.

the mitochondrial protein transport system. Its proper function and activity are important to ensure efficient and proper import of proteins into the mitochondria,

Table 7. Results of Lung Mitochondrial Biogenesis Coefficient of Variation on PGC-1α Component

Component	Group	Average	Standard Deviation	Total	Coefficient of Variance
PGC-1α	F: Exercise	0.639515	0.249651	5	39.03758
PGC-1α	I: Exercise + Moringa + Pollution	0.750907	0.257544	5	34.29776
PGC-1α	G: Exercise + Pollution	0.788075	0.243538	5	30.90284
PGC-1α	C: No exercise + Pollution	0.680282	0.194925	5	28.65353
PGC-1α	D: No exercise + Vitamin C	0.732361	0.18551	5	25.33035
PGC-1α	H: Exercise + Moringa	0.81664	0.197068	5	24.13162
PGC-1α	E: No exercise + Moringa + Pollution	0.684202	0.148858	5	21.75643
PGC-1α	B: No exercise + Moringa	0.73898	0.144279	5	19.52412
PGC-1α	A: Without exercise	0.74539	0.109111	5	14.63817
	Total	0.730706			

Table 8. Results of Lung Mitochondrial Biogenesis Coefficient of Variation on TOM20 Component

Component	Group	Average	Standard Deviation	Total	Coefficient of Variance
TOM20	C: No exercise + Pollution	0.760234	0.26108	5	34.34209
TOM20	I: Exercise + Moringa + Pollution	0.716411	0.167047	5	23.31721
TOM20	D: No exercise + Vitamin C	0.720585	0.151717	5	21.05465
TOM20	A: Without exercise	0.847977	0.177692	5	20.95476
TOM20	B: No exercise + Moringa	0.869899	0.167072	5	19.20588
TOM20	F: Exercise	0.780693	0.148528	5	19.02512
TOM20	E: No exercise + Moringa + Pollution	0.787496	0.147905	5	18.78173
TOM20	H: Exercise + Moringa	0.904782	0.150062	5	16.58547
TOM20	G: Exercise + Pollution	0.864325	0.088523	5	10.24185
	Total	0.805822			

Table 9. Results of Lung Mitochondrial Biogenesis Coefficient of Variation on COX IV Component

Component	Group	Average	Standard Deviation	Total	Coefficient of Variance
COX IV	I: Exercise + Moringa + Pollution	0.762574	0.238688	5	31.30029
COX IV	C: No exercise + Pollution	0.767999	0.210607	5	27.42283
COX IV	E: No exercise + Moringa + Pollution	0.742661	0.162017	5	21.81568
COX IV	H: Exercise + Moringa	0.89954	0.177492	5	19.73147
COX IV	A: Without exercise	0.898299	0.152493	5	16.97576
COX IV	F: Exercise	0.800404	0.135243	5	16.89686
COX IV	B: No exercise + Moringa	0.88378	0.128763	5	14.56953
COX IV	D: No exercise + Vitamin C	0.728808	0.100051	5	13.728
COX IV	G: Exercise + Pollution	0.87614	0.092057	5	10.5071
	Total	0.817801			

Exercise provides a good value on the formation of TOM20 protein located on the mitochondrial outer membrane and is one of the important components in

thus affecting the function and balance of mitochondria in the cell.

The high COX IV (Cytochrome C Oxidase Subunit IV) value in a subject is generally indicated as some-

thing favorable or positive. A high COX IV value indicates that the subject has a greater level or amount of cytochrome oxidase enzyme in the mitochondria. Administration of moringa and vitamin C did not have an effect on increasing the amount of cytochrome oxidase enzyme found in mitochondria biogenesis.

DISCUSSION

Research findings presented that Moringa administration with IL-6 and NF-κB consistently decreased lung inflammation. This might occur due to the following reasons, such as (1) Moringa acts as a natural anti-inflammatory, as moringa has been known to have strong anti-inflammatory properties that can help reduce inflammation in the body (Howladar, 2014; Kasolo, 2010); Moringa oleifera leaf extract contains anti-inflammatory compounds such as isothiocyanate and polyphenols, which can reduce the production of pro-inflammatory cytokines and other inflammatory mediators (Cheenpracha, 2010; Jaja-Chimedza, 2017); (2) IL-6 as an inflammatory mediator, IL-6 is a pro-inflammatory cytokine produced by various cells in the body in response to injury or infection. Overproduction of IL-6 can lead to chronic inflammation, including inflammation of the lungs. Therefore, administering Moringa together with IL-6 can inhibit the action of excessive IL-6 and thus reduce inflammation (Nomoto et al., 2009); (3) NF-κB as an inflammatory regulator, NF-κB is a protein that plays a role in regulating inflammatory genes and immune responses in the body (Hoesel, 2013; Iliopoulos, 2009). Excessive activation of NF-κB can cause chronic inflammation and damage lung tissue. Studies have shown that several compounds found in Moringa can inhibit NF-κB activation, thus helping reduce lung inflammation; and (4) potential synergy, the combination of Moringa administration with IL-6 and NF-κB can have a synergistic effect in reducing lung inflammation (Ben-Neriah, 2011). By acting at different levels of the inflammatory pathway, the three elements can complement each other and enhance the anti-inflammatory effect.

The findings of this study indicate that Moringa with IL-6 and NF-κB can provide a good inflammatory impact and does not cause excessive or chronic inflammation (Franceschi, 2014; Landskron, 2014; Xu, 2003). Because excessive or chronic inflammation can be a sign or risk factor for various diseases, including autoimmune diseases (rheumatoid arthritis, lupus, and Crohn's disease), cardiovascular diseases (heart disease, atherosclerosis, and stroke), metabolic diseases (obesity, type 2 diabetes, and metabolic syndrome), respiratory diseases (asthma, chronic bronchitis, and chronic obstructive pulmonary disease (COPD)), neurological diseases (Alzheimer's and Parkinson's disease), gastrointestinal disorders (ulcerative colitis or Crohn's disease), cancer, skin diseases (psoriasis, eczema, and acne), as well as disorders of mental and emotional well-being (such as mood disorders and anxiety disorders) (Li & Chung, 2016; Ruby, 1995). As such, lower inflammation in subjects is considered better as it is associated with better health and reduced risk of chronic diseases. But even so, any assessment of inflammation should be done in a clinical context and consider other factors relevant to the condition of the subject being observed.

Furthermore, administration of Moringa and exercise has a good impact on PGC-1α and stimulates the formation of mitochondrial (Zong, 2002) indicated by the average value of large mitochondrial. This can occur because Moringa has several active compounds that can affect metabolic processes in cells, while exercise can increase energy demand which can trigger changes at the cellular level. In addition, moringa can also be a source of nutrients, including vitamins, minerals, proteins, and phytochemical compounds such as flavonoids, polyphenols, and isothiocyanates. These compounds have been known to have antioxidant and anti-inflammatory activities, which can help protect cells from oxidative stress and inflammation. Then, PGC-1α is a transcription factor that plays a role in regulating the expression of genes involved in energy metabolism and the formation of mitochondria, the cell organelles responsible for generating energy in the form of ATP (Adenosine Triphosphate) through the oxidation of glucose and fatty acids (Ohta, 2001). PGC-1α plays a role in regulating mitochondrial biogenesis, the process of forming new mitochondria in cells (Moyes, 1997).

During exercise, the body requires more energy to carry out physical activities. This increased energy demand can trigger various adaptive responses in cells, including increased expression of PGC-1α. Physical activity also increases the production of ROS (Reactive Oxygen Species) or free radicals which are by- products of aerobic metabolism. This may stimulate antioxidant

responses in cells that may contribute to the positive regulation of PGC-1α (Mittal, 2014; Nimse, 2015). Furthermore, the combination of moringa administration and exercise can provide a positive synergy. The nutrient content and active compounds in moringa may help protect cells from oxidative stress and relieve inflammation, thus allowing cells to function more efficiently and respond better to exercise stimuli. Exercise can increase the effectiveness and efficiency of nutrient utilization from food, including the beneficial compounds in Moringa. Thus, a large mitochondrial biogenesis value is generally considered favorable as it can improve energy efficiency, healthy metabolism, oxidative protection, and physical adaptation (Wu, 2020).

In addition to providing good benefits to PGC-1α and stimulating mitochondrial formation, based on research findings, exercise can also provide good value to the formation of TOM20 protein which is located on the mitochondrial outer membrane and is one of the important components in the mitochondrial protein transport system. Exercise has been shown to stimulate mitochondrial biogenesis. By increasing the number of mitochondria in the cell, the opportunity for TOM20 protein formation also increases. In addition, during exercise, physical activity causes an increase in AMP (Adenosine Monophosphate) levels in cells. AMPK (AMP-activated Protein Kinase) is an enzyme activated by AMP playing a role in regulating energy metabolism. AMPK activation can increase the transcription of genes involved in mitochondrial biogenesis and the expression of mitochondrial proteins such as TOM20 (Dominy, 2013).

Exercise increases cellular energy requirements, meaning that there is increased oxidation of glucose and fatty acids in the mitochondria to produce ATP. This oxidation process may affect the positive regulation of mitochondrial proteins, including the TOM20 protein, as mitochondria plays a central role in cellular energy metabolism. In addition, regular exercise is also known to enhance the body natural antioxidant system and can reduce oxidative stress which can help protect mitochondria from oxidative damage and maintain healthy mitochondrial function, so that it can also affect the expression of mitochondrial proteins including TOM20 (Chen, 2011; Nguyen, 2009). Therefore, exercise has been shown to have an effect on gene expression in the body. Several studies have also shown that exercise can affect the expression of genes related to energy metabolism and mitochondrial biogenesis, which may contribute to the positive regulation of mitochondrial proteins such as TOM20.

Research findings represent the enzyme cytochrome oxidase, which is involved in the mitochondrial respiration chain, to have an important role in the process of cellular energy production in mitochondria. The high expression level of COX IV component could indicate an increase in the activity and amount of cytochrome oxidase in the mitochondria. It could be a sign of increased cellular capacity to produce energy through the respiration process. Based on the results of a study, increased COX IV expression has been associated with various biological conditions and responses, the first is an increased metabolic state in response to higher energy demands, such as during intense exercise and hypoxic (oxygen deprivation) (Yin, 2008), or increased energy demand during growth and development. The second, mitochondrial adaptation showing high COX IV expression levels can indicate mitochondrial adaptation to oxidative stress or increased metabolic activity (Kurutas, 2016). This could occur in response to exposure to pollutants, altered environmental conditions, or certain diseases. The third, metabolic disorders representing increased COX IV expression has also been associated with several metabolic diseases and disorders, such as diabetes, obesity, and neurodegenerative diseases (Golpich, 2017).

Based on the presented findings and discussion, it concludes that there was a differentiation in the level of inflammation and mitochondrial lung biogenesis from the administration of Moringa oleifera leaf extract on IL -6 and NF-κB biomarkers in physical activities, indicated by a comparison of the average value of the inflammatory process and lung mitochondrial biogenesis in nine treatment groups. The results showed the following results, (1) higher IL-6 levels occurred in the exercise group accompanied by pollution (Group G) and the Exercise Group in open space without pollution (Group F); (2) the average result showed that the largest NF-κB component was in Group C and the smallest was in Group I; (3) the NF-κB component was high in the group without exercise accompanied by air pollution and tended to be low in the group with Moringa administration, showing that Moringa administration could reduce the level of inflammation in both polluted

and non-polluted outdoor exercise groups; (4) the average results showed that the largest PGC-1α component was in group H and the smallest was in group F, the findings showed that the IL-6 component tended to be high; (5) the average results showed that the largest TOM20 component was in group H and the smallest was in group I, the findings showed that the TOM20 component tended to be high; and (6) the average results showed that the largest COX IV component was in group H and the smallest was in group D, the findings showed that the COX IV component tended to be high.

CONCLUSION

Based on the findings and discussion, it concludes that (1) administration of Moringa with IL6 and NF-κB consistently reduced lung inflammation; (2) a large mitochondrial biogenesis value is generally considered favorable because it can improve energy efficiency, healthy metabolism, oxidative protection, and physical adaptation; (3) exercise provides a good value on the formation of TOM20 protein located on the mitochondrial outer membrane and is one of the important components in the mitochondrial protein transport system; and (4) a high COX IV value indicates that the subject has a greater level or amount of cytochrome oxidase enzyme in the mitochondria.

Despite the best efforts, the study still has some limitations, both in terms of process and methodology. In this study, physical activity outside the treatment could not be strictly controlled and monitored, allowing rats to vary their activities. This condition might affect the volume of physical activity of each rat in both the exercise and no exercise groups. The research subjects used male Wistar rats which, in some conditions, have limitations with the real participants being measured, namely humans. The study still involved a limited sample, short-term monitoring, and the use of relatively simple lung monitoring technology.

Based on these conclusions, although Moringa and its combination with IL-6 and NF-κB can consistently reduce inflammation in mice, the potential of Moringa and its combination with IL-6 and NF-κB in reducing inflammation, especially in humans, still requires further research to ensure its clinical effectiveness and safety.

ACKNOWLEDGEMENT

Bionanotechnology Laboratory, Institute of Research and Community Service, Universitas Pendidikan Indonesia.

REFERENCES

Albert, M. A., Danielson, E., Rifai, N., Ridker, P. M., Prince Investigators, & PRINCE Investigators. (2001). Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *Jama*, *286*(1), 64-70.

Ben-Neriah, Y., & Karin, M. (2011). Inflammation meets cancer, with NF-κB as the matchmaker. *Nature immunology*, *12*(8), 715-723.

Calvano, S. E., Xiao, W., Richards, D. R., Felciano, R. M., Baker, H. V., Cho, R. J., ... & Inflammation and Host Response to Injury Large Scale Collaborative Research Program. (2005). A network-based analysis of systemic inflammation in humans. *Nature*, *437* (7061), 1032-1037.

Cheenpracha, S., Park, E. J., Yoshida, W. Y., Barit, C., Wall, M., Pezzuto, J. M., & Chang, L. C. (2010). Potential anti-inflammatory phenolic glycosides from the medicinal plant Moringa oleifera fruits. *Bioorganic & medicinal chemistry*, *18*(17), 6598-6602.

Chen, S. D., Yang, D. I., Lin, T. K., Shaw, F. Z., Liou, C. W., & Chuang, Y. C. (2011). Roles of oxidative stress, apoptosis, PGC-1α and mitochondrial biogenesis in cerebral ischemia. *International journal of molecular sciences*, *12*(10), 7199-7215.

Creswell, J. W., & Clark, V. L. P. (2017). *Designing and conducting mixed methods research*. Sage publications.

Dominy, J. E., & Puigserver, P. (2013). Mitochondrial biogenesis through activation of nuclear signaling proteins. *Cold Spring Harbor perspectives in biology*, *5*(7), a015008.

Elias, R. J., Kellerby, S. S., & Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Critical reviews in food science and nutrition*, *48*(5), 430-441.

Elinav, E., Nowarski, R., Thaiss, C. A., Hu, B., Jin, C., & Flavell, R. A. (2013). Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nature Reviews Cancer*, *13*(11), 759-771.

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution

to age-associated diseases. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *69*(Suppl_1), S4-S9.

Freeman, W. H. (2013). *Physical education, exercise and sport science in a changing society*. Jones & Bartlett Publishers.

Golpich, M., Amini, E., Mohamed, Z., Azman Ali, R., Mohamed Ibrahim, N., & Ahmadiani, A. (2017). Mitochondrial dysfunction and biogenesis in neurodegenerative diseases: pathogenesis and treatment. *CNS neuroscience & therapeutics*, *23*(1), 5-22.

Gureev, A. P., Shaforostova, E. A., & Popov, V. N. (2019). Regulation of mitochondrial biogenesis as a way for active longevity: interaction between the Nrf2 and PGC-1α signaling pathways. *Frontiers in genetics*, *10*, 435.

Hagger, M. S., & Smith, B. (2018). Research approaches in the sport, exercise, and physical activity field. *Advances in sport and exercise psychology*, 17-36.

Heinonen, S., Buzkova, J., Muniandy, M., Kaksonen, R., Ollikainen, M., Ismail, K., ... & Pietiläinen, K. H. (2015). Impaired mitochondrial biogenesis in adipose tissue in acquired obesity. *Diabetes*, *64*(9), 3135-3145.

Hoesel, B., & Schmid, J. A. (2013). The complexity of NF-κB signaling in inflammation and cancer. *Molecular cancer*, *12*(1), 1-15.

Howladar, S. M. (2014). A novel Moringa oleifera leaf extract can mitigate the stress effects of salinity and cadmium in bean (Phaseolus vulgaris L.) plants. *Ecotoxicology and Environmental Safety*, *100*, 69-75.

Iliopoulos, D., Hirsch, H. A., & Struhl, K. (2009). An epigenetic switch involving NF-κB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell*, *139*(4), 693-706.

Jaja-Chimedza, A., Graf, B. L., Simmler, C., Kim, Y., Kuhn, P., Pauli, G. F., & Raskin, I. (2017). Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (Moringa oleifera) seed extract. *PloS one*, *12*(8), e0182658.

Kasolo, J. N. (2010). Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities. *Journal of Medicinal Plants Research*, *4*(9), 753–757.

Kirschner, H., Kuyken, W., Wright, K., Roberts, H., Brejcha, C., & Karl, A. (2019). Soothing your heart and feeling connected: A new experimental paradigm to study the benefits of self-compassion. *Clinical Psychological Science*, *7*(3), 545-565.

Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition journal*, *15*(1), 1-22.

Landskron, G., De la Fuente, M., Thuwajit, P., Thuwajit, C., & Hermoso, M. A. (2014). Chronic inflammation and cytokines in the tumor microenvironment. *Journal of immunology research*, *2014*.

Li, W. H. C., & Chung, O. K. J. (2016). Promoting regular physical activity among childhood cancer survivors using Adventure-based Training. In *Conference on Optimizing Healthcare Quality: Teamwork in Education, Research, & Practice*.

Mileva, K. N., & Zaidell, L. (2022). Sport and Exercise Science and Health. In *Health studies: An introduction* (pp. 85-124). Singapore: Springer Singapore.

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxidants & redox signaling*, *20*(7), 1126-1167.

Moyes, C. D., Mathieu-Costello, O. A., Tsuchiya, N., Filburn, C., & Hansford, R. G. (1997). Mitochondrial biogenesis during cellular differentiation. *American Journal of Physiology-Cell Physiology*, *272*(4), C1345-C1351.

Nguyen, T., Nioi, P., & Pickett, C. B. (2009). The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *Journal of biological chemistry*, *284*(20), 13291-13295.

Nimse, S. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances*, *5* (35), 27986–28006. https://doi.org/10.1039/c4ra13315c

Nomoto, T., Okada, T., Shimazaki, K., Yoshioka, T., Nonaka-Sarukawa, M., Ito, T., ... & Ozawa, K. (2009). Systemic delivery of IL-10 by an AAV vector prevents vascular remodeling and end-organ damage in stroke-prone spontaneously hypertensive rat. *Gene therapy*, *16*(3), 383-391.

Ohta, A., & Sitkovsky, M. (2001). Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature*, *414*(6866), 916-920.

De Oliveira, M. R., Nabavi, S. F., Manayi, A., Daglia, M., Hajheydari, Z., & Nabavi, S. M. (2016). Resveratrol and the mitochondria: From triggering the intrinsic apoptotic pathway to inducing mitochondrial biogenesis, a mechanistic view. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1860*(4), 727-745.

Pfanner, N., Warscheid, B., & Wiedemann, N. (2019). Mitochondrial proteins: from biogenesis to functional networks. *Nature reviews Molecular cell biology*, *20*(5), 267-284.

Ruby, A. J., Kuttan, G., Babu, K. D., Rajasekharan, K. N., & Kuttan, R. (1995). Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer letters*, *94*(1), 79-83.

Rüsch, N., Angermeyer, M. C., & Corrigan, P. W. (2005). Mental illness stigma: Concepts, consequences, and initiatives to reduce stigma. *European psychiatry*, *20*(8), 529-539.

Smith, B., Williams, O., Bone, L., & Collective, T. M. S. W. C. P. (2023). Co-production: A resource to guide co-producing research in the sport, exercise, and health sciences. *Qualitative Research in Sport, Exercise and Health*, *15*(2), 159-187.

Stehling, O., Wilbrecht, C., & Lill, R. (2014). Mitochondrial iron–sulfur protein biogenesis and human disease. *Biochimie*, *100*, 61-77.

Wu, L., Li, L., Chen, S., Wang, L., & Lin, X. (2020). Deep eutectic solvent-based ultrasonic-assisted extraction of phenolic compounds from Moringa oleifera L. leaves: Optimization, comparison and antioxidant activity. *Separation and Purification Technology*, *247*, 117014.

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., ... & Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *The Journal of clinical investigation*, *112*(12), 1821-1830.

Yin, W., Signore, A. P., Iwai, M., Cao, G., Gao, Y., & Chen, J. (2008). Rapidly increased neuronal mitochondrial biogenesis after hypoxic-ischemic brain injury. *Stroke*, *39*(11), 3057-3063.

Zhang, H., Gao, P., Fukuda, R., Kumar, G., Krishnamachary, B., Zeller, K. I., ... & Semenza, G. L. (2007). HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. *Cancer cell*, *11*(5), 407-420.

Zong, H., Ren, J. M., Young, L. H., Pypaert, M., Mu, J., Birnbaum, M. J., & Shulman, G. I. (2002). AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proceedings of the national academy of sciences*, *99*(25), 15983-15987.