

Exercise training is more effective than resveratrol supplementation on alleviation of inflammation in peritoneal macrophages of high fat diet mice

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[Purpose] This study investigated the effect of exercise training and resveratrol supplementation with low fat diet on proinflammatory profiles by Lipopolysaccharide (LPS)-stimulation in peritoneal macrophage of high fat diet mice. **[Methods]** To accomplish the purpose of this study, C57BL/6 male mice were fed high fat diet (45% fat diet) for 8 weeks. Then these mice were divided into 3 groups; HLC (high fat diet and low fat diet for 8 weeks as the control, n=10), HLR (high fat diet and low fat diet for 8 weeks with resveratrol supplementation, n=10). HLE (high fat diet and low fat diet for 8 weeks with moderate exercise training, n=10). Resveratrol (10 mg/kg) was administrated once a day, 5 days/week for 8 weeks. Exercise training was performed for 8 weeks on a treadmill running for 30-60 min/day at 10-22 m/min, 0% grade, 5 days/week. After exercise training, all the peritoneal macrophage was collected and LPS (0, 0.5, 1.0 µg/ml) were used to stimulate the cells. Then peritoneal macrophage TNF-α, IL-6, MCP-1, IL12p70, IFN-γ, IL-10 were measured by BD cytometric bead array mouse inflammation kit. **[Results]** As a result, body weight and total cholesterol were significantly reduced in HLE compared with HLC (p<.05). Also, TNF-α and MCP-1 were decreased in HLE compared with HLC (p<.05) by LPS-stimulation (0, 0.5, 1.0 µg/ml) and IL-6, IL-12p70 and IFN-γ were decreased in HLE compared with HLC (p<.05) by LPS-stimulation (1.0 µg/ml). But resveratrol supplementation did not affect the result. **[Conclusion]** These findings suggest that exercise training has beneficial effects on body weight, total cholesterol, peritoneal macrophage and proinflammatory cytokine in high fat diet mice. **[Keyword]** High-fat diet; resveratrol; moderate exercise; macrophage; inflammation

INTRODUCTION

Chronic obesity from lack of physical activity or high fat diet is a cause for cardiovascular disease, metabolic syndrome, arteriosclerosis, type-2 diabetes and immune function decrease [1] by sustaining the low level of inflammation.

Especially, excessive fat accumulation due to high fat diet is known to cause the increase of inflammation level and brings negative effects on sensitivity and activity of immune cells toward exogenous antigen by direct and indirect influence on metabolic process and endocrine system [2].

Macrophage is an important defense mechanism for innate immunity and it does nonspecific phagocytosis and digestion of bacteria and foreign body in the body and during such process, it regulates immunity by secreting cytokines [3].

Also excessively secreted proinflammatory cytokine from macrophage such as TNF-α (tumor necrosis factor α), IL-6 (interleukin 6), CRP (C-reactive protein), MCP-1 (monocyte

chemoattractant protein 1) are known to directly affect the onset of insulin resistance, the prevalence of diabetes and atherosclerosis of coronary arteries [4].

Previous studies on obesity and the immunity of macrophage reported imbalance of control between proinflammatory cytokines and anti-inflammatory cytokines due to the decrease of macrophage's phagocytosis or stimulation of antigen in obese mice [5,6] and considering the improvement of macrophage's phagocytosis and reduced expression-levels of nitric oxide caused by low fat diet on the obese model [7], obesity appears to be the cause of the damage of innate immunity or activity decrease toward exogenous antigen.

TNF-α, IL-1β and MCP-1, which control the inflammation level of the body, have important roles in inflammation-control network and they increase when infection or invasion of antigen occurs, and IL-6 is known to control CRP-synthesis and inflammation-induction and IFN-γ is known to affect on the synthesis of inflammatory cytokines and Amar *et al.* [8]

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reported that the imbalance of immune system in obese mice from high fat diet was occurred due to increased arteriosclerosis factors and increased expression of proinflammatory cytokine such as TNF- α , IL-1 β , IL-6 and IL-12 in immune cells, which was stimulated by LPS.

Conducting medium strength exercises brings positive effects of proinflammatory cytokine decrease, which was expressed from immune cells, and immunity increase in the obese model [9,10] and even with moderate one-off exercise alone, chemotaxis increase of macrophage [11] and significant results on lymphocyte proliferation response and antigen-specific immune response, which were gained upon the completion of mitogen stimulation with antigen, were achieved [12], but high-intensity one-off exercise or exhaustive exercise was reported to negatively affect immunity by increasing contagiousity [13] and proinflammatory cytokine such as IL-6 and IL-1ra and thus the results are contradictory based on exercise intensity or the duration of exercise.

Especially, most people recognize the method of combining medium strength exercises with diet-restriction (e.g. low fat diet and low calorie diet) as an ideal method for improving the issue of obesity and thus studies on the effects of medium strength exercises combined with low fat diet for the obesity model on the weight reduction or anti-inflammatory function is very meaningful.

Resveratrol (3,5,4'-trihydroxystilbene) is a representative type of polyphenol complex and can be found in a large quantity in fruit, peanut and grape-skin and it is known to have biochemical and physiological effects in the body including anti-inflammatory activity, antioxidant activity, anti-cardiovascular disease, anti-cancer, etc.

It was reported recently from a model, induced by hyperlipidemia, that it shows effects on inhibition of inflammation, NF-kappaB (NF-kB) decrease in diabetes and positive change on lipid metabolism and prevention of non-alcoholic fatty liver [14].

Also, mitochondria function increase by increasing the energy consumption of muscle and brown adipose tissue in high fat diet mice was reported and thus it suggested that it has positive effects on weight reduction which is directly related to inflammation level [15].

Recently various studies have been conducted on calorie-restriction [16] or various exercise methods with diverse intensity or health supplement intake (e.g. polyphenol complex) as methods for reducing chronic inflammation level and improving decreased-immune function but rare are the studies dealing with conducting low fat diet combined with polyphenol complex and medium strength exercises and their effects on macrophage on the obese model, fattened by high fat diet,

which plays an important role for initial immune function and controlling inflammation level in the body.

Therefore, this study was aimed to reveal the effects of resveratrol intake combined with low fat diet and medium strength exercises in the obese model on the improvement of obesity and the expression of proinflammatory cytokine, which is expressed from immune cells, by studying the change pattern of proinflammatory cytokine, expressed after the stimulation by LPS (Lipopolysaccharide), which is antigen-presenting material, on macrophage obtained from abdominal cavity.

METHODS

Animals and diet

A total of 30, C57BL / 6 type, male mice of 5-weeks-old were received from Joong Ang laboratory animal company and after 1 week of adjustment period in laboratory, high fat diet was given for 8 weeks to be freely eaten to induce obesity [17].

After inducing obesity, they were categorized into a control group (HLC, High fat diet-low fat diet control, n=10), a resveratrol group (HLR, High fat diet-low fat diet with resveratrol supplementation, n=10) and an exercise group (HLE, High fat diet-low fat diet with exercise, n=10) and then resveratrol administration and exercise were applied for 8 weeks.

The average temperature of the breeding room for laboratory mice were 22 ± 1 Celsius with humidity of $60 \pm 5\%$ and day/night cycle of laboratory animal center was automatically adjusted per 12 hours.

Feed and water were supplied amply and experiments were conducted following the law on laboratory animal treatments and the permission from Animal experimentation ethics committee of Chungnam National University was obtained (CNU-00202).

High fat diet was conducted with high-fat feed according to a previous study [4].

Table 1. Formulas of rodent feed

Product (#)	High fat Diet(D12451)		Low fat diet(D12450)	
	g%	kcal%	g%	kcal%
Protein	24	20	19.2	20
Carbohydrate	41	35	67.3	70
Fat	24	45	4.3	10
Total		100		100
kcal/gm	4.73		3.85	

For feeding, high-fat feed (45% fat, rodent diet with high fat, #D12451) and low-fat feed (10% fat rodent diet with low fat, #D12450) were purchased from Orient Bio company and for the record of feed intake, 20 g was given once per day at the same time and the remainder of feed was measured to get the number and the weight of mice were measured once per week at the same time and the composition of diet is listed in <Table 1>.

Resveratrol supplementation

Resveratrol (Sigma Aldrich, St Louis, MO) was dissolved in Dimethyl Sulfoxide (DMSO) solution and then measured by 10 mg/(d × kg body wt) and given orally at the same time of each day to a concerning group, with the ratio of 0.1 ml of the solution per mouse, which had become obese due to 8 weeks of high fat diet and only DMSO solution of the same quantity was given to non-resveratrol group.

Exercise protocol

1 week of adaptation period was provided for treadmill exercise (with the speed of 8 m/min).

A warm up exercise for all the exercises was conducted for 5~10 minutes with the speed of 8-10 m/min and the main exercise was done with the speed of 10-22 m/min for 30~60 minutes and it was composed using the principle of cumulative overload.

This speed is about 60-76% of maximum oxygen consumption of mice and such score means it was medium strength exercises [18].

Regarding exercise frequency, it was conducted 5 days in a row per week.

During exercise of the exercise-group, the control group was also exposed to the same environmental stress and the noise and vibration of the treadmill and feed and water supply were limited as well.

To maximally reduce exercise-related stress during exercise, no outside stimulation or electric shock was applied during exercise.

An experiment diary was recorded daily and an assessment of test animal was done by the people who are fully educated and well-equipped to handle them with education and practice before the treadmill exercise.

Preparation of peritoneal macrophages

3% thioglycollate (DIFCO, USA) 2 ml was injected into the abdominal cavity of mouse and induced macrophage 3

days before the sacrifice.

After anesthesia, 5 ml of RPMI 1640 medium (GIBCO, USA) was inserted into the abdominal cavity and a massage was gently applied for 2~3 minutes and then peritoneal cell was collected using 5 ml syringe and the process was repeated 3 times and collected about 10-15 ml of peritoneal cell.

The number of cells for collected peritoneal macrophages was checked using Trypan blue dye, and they were diluted to a concentration of 1×10^6 cells/ml and cell-liquid was dispensed into 24 well plate (NUNC, Denmark) and cultivated for 2 hours in a 37 °C, 5% CO₂ cell culture incubator and then non-adherent cells were removed while swapping FBS-containing medium and antibiotics.

Blood sample collection and analysis

To prevent temporary effects on macrophage and blood sample, resveratrol and exercise were discontinued 24 hours before the sampling and the feed was discontinued 12 hours before the sampling.

The animals were anesthetized and then abdominal incision was conducted and blood was collected from the inferior vena cava and then the extract was centrifuged and plasma was stored frozen at -70°C.

Triglyceride, total cholesterol and low density lipoprotein cholesterol were analyzed using Fuji 7000i automatic biochemical analysis system (Fujifilm, Japan).

Measurement of proinflammatory cytokine

The liquid was dispensed into each well of microplate (Corning Laboratory Science Co., USA) with 1×10^6 of cell per each and cell culture medium, which was treated with Lipopolysaccharide of the concentration of 0, 0.5, 1.0 µg/ml, was collected 24 hours later and then TNF- α, IL-6, MCP-1, IL-12p70, IFN- γ and IL-10 were analyzed using BD Cytometric Bead Array Mouse Inflammation Kit (BD Sciences, USA).

To sum up the assessment method briefly, it was conducted by dissolving standard reagent in diluent buffer 2 ml and then incubated for 15 min at room temperature and had serial dilution with the ratio of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:356.

Mixed capture bead was dispensed into standard tube and sample tube by 50µl per each and 50µl of detection antibody was added and light tapping was applied and then incubated at room temperature for 2 hours.

Washing buffer 1 ml was added and then it was centrifuged at 12,000 rpm for 5 minutes.

300 μ l of washing buffer was added and then it was measured using FACs Canto II (Becton, Dickinson, USA).

Statistical methods

All the data were calculated into descriptive statistics and were presented as mean and standard deviation and SPSS 18.0 statistical analysis was used for the statistical data and ANOVA (One way ANOVA) was used for testing body weight between the groups, blood lipid variables and the significance of the difference between average expression levels of proinflammatory cytokine and then Tukey test was used for post hoc test.

Significance level was set at $p = .05$.

RESULTS

Changes of body weight, lipid profiles

Upon the completion of 8 weeks of low fat diet combined with resveratrol administration and medium strength exercises on the obese mice, which were fattened with 8 weeks of high fat diet, weight was reduced in HLE group ($p = .017$, Table 2) compared to HLC and HLR group and regarding blood

lipid variables, total cholesterol was significantly reduced in HLE group ($p = .015$, Table 2) compared to HLC group.

TNF- α production

Upon the completion of 8 weeks of low fat diet combined with resveratrol intake and medium strength exercises on the obese mice, which were fattened with 8 weeks of high fat diet, TNF- α expression level of peritoneal macrophages was significantly decreased in HLE group compared to HLC group for both of non-LPS-stimulation (0 μ g/ml) or LPS-stimulation with different concentration (0.5 μ g/ml, 1.0 μ g/ml) ($p = .026$, $p = .021$, $p = .030$, Table 3).

IL-6 production

Upon the completion of 8 weeks of low fat diet combined with resveratrol administration and medium strength exercises on the obese mice, which were fattened with 8 weeks of high fat diet, IL-6 expression level of peritoneal macrophages was different based on the concentration of LPS.

There was no significant difference among groups when LPS was not applied (0 μ g/ml) or applied with low concentration (0.5 μ g/ml), but when high concentration (1.0 μ g/ml) of LPS was applied, HLR group and HLE groups had

Table 2. Comparison of body weight and plasma lipid profiles in each group

Group	HLC	HLR	HLE	F	p
Body weight (g)	41.97 \pm 2.36	41.87 \pm 2.67	38.62 \pm 2.68*	4.864	.017
TG (mg/dl)	83.01 \pm 24.39	68.50 \pm 11.66	79.62 \pm 10.38	2.223	.143
TC (mg/dl)	195.14 \pm 13.18	196.00 \pm 25.63	157.50 \pm 29.18*	5.535	.015
LDL-C (mg/dl)	125.88 \pm 58.97	137.18 \pm 26.14	106.40 \pm 4.44	.805	.465

Values are presented as mean \pm SD. n = 10. HLC, high fat diet-low fat diet control; HLR, high fat diet-low fat diet with resveratrol supplementation; HLE, high fat diet-low fat diet with exercise training. TG, triglyceride; TC, Total cholesterol; LDL-C, Low density lipoprotein cholesterol. * $P < .05$ versus HLC. † $P < .05$ versus HLR.

Table 3. Comparison of TNF- α production in each group

Group	HLC	HLR	HLE	F	p
LPS non Stimulated (pg/ml)	58.36 \pm 8.30	53.89 \pm 11.47	40.77 \pm 6.97*	5.026	.026
LPS-stimulated (0.5 μ g/ml) (pg/ml)	228.97 \pm 37.19	210.18 \pm 45.18	156.52 \pm 21.32*	5.464	.021
LPS-stimulated (1.0 μ g/ml) (pg/ml)	372.19 \pm 106.98	288.31 \pm 99.92	198.87 \pm 48.22*	4.743	.030

Values are presented as mean \pm SD. n = 10. HLC, high fat diet-low fat diet control; HLR, high fat diet-low fat diet with resveratrol supplementation; HLE, high fat diet-low fat diet with exercise training. * $P < .05$ versus HLC.

Table 4. Comparison of IL-6 production in each group

Group	HLC	HLR	HLE	F	p
LPS non Stimulated (pg/ml)	52.05 \pm 20.60	61.78 \pm 18.04	40.78 \pm 10.12	1.943	.186
LPS-stimulated (0.5 μ g/ml) (pg/ml)	73.80 \pm 33.27	89.84 \pm 28.16	56.56 \pm 14.84	1.959	.184
LPS-stimulated (1.0 μ g/ml) (pg/ml)	528.69 \pm 131.51	316.05 \pm 100.26*	255.29 \pm 69.40*	9.610	.003

Values are presented as mean \pm SD. n = 10. HLC, high fat diet-low fat diet control; HLR, high fat diet-low fat diet with resveratrol supplementation; HLE, high fat diet-low fat diet with exercise training. * $P < .05$ versus HLC.

significantly lower score ($p = .003$, Table 4) compared to HLC group.

MCP-1 production

Upon the completion of 8 weeks of low fat diet combined with resveratrol intake and medium strength exercises on the obese mice, which were fattened with 8 weeks of high fat diet, MCP-1 expression level of peritoneal macrophages was the same with the case of TNF- α that it was significantly reduced in HLE group ($p = .013$, $p = .006$, $p = .005$) compared to non-LPS-stimulation group (0 $\mu\text{g/ml}$) and LPS-stimulation group with different concentration usage (0.5 $\mu\text{g/ml}$, 1.0 $\mu\text{g/ml}$).

Also, HLE group had significantly reduced score compared to HLR group when the concentration of LPS-stimulation was 1.0 $\mu\text{g/ml}$.

IL-12p70 production

Upon the completion of 8 weeks of low fat diet combined

Table 5. Comparison of MCP-1 production in each group

Group	HLC	HLR	HLE	F	p
LPS non Stimulated (pg/ml)	13.39 \pm 3.28	11.80 \pm 3.11	7.57 \pm 0.90*	6.361	.013
LPS-stimulated (0.5 $\mu\text{g/ml}$) (pg/ml)	22.85 \pm 4.90	18.07 \pm 5.95	11.12 \pm 2.07*	8.182	.006
LPS-stimulated (1.0 $\mu\text{g/ml}$) (pg/ml)	35.03 \pm 9.60	32.41 \pm 9.11	15.44 \pm 5.40* [†]	8.292	.005

Values are presented as mean \pm SD. n = 10. HLC, high fat diet-low fat diet control; HLR, high fat diet-low fat diet with resveratrol supplementation; HLE, high fat diet-low fat diet with exercise training. * $P < .05$ versus HLC. [†] $P < .05$ versus HLR.

Table 6. Comparison of IL-12p70 production in each group

Group	HLC	HLR	HLE	F	p
LPS non Stimulated (pg/ml)	15.45 \pm 3.62	14.42 \pm 2.45	12.48 \pm 1.27	1.637	.235
LPS-stimulated (0.5 $\mu\text{g/ml}$) (pg/ml)	13.84 \pm 2.41	13.07 \pm 2.05	12.21 \pm 1.37	.838	.456
LPS-stimulated (1.0 $\mu\text{g/ml}$) (pg/ml)	20.04 \pm 15.10	15.10 \pm 2.12	11.00 \pm 2.88*	9.551	.003

Values are presented as mean \pm SD. n = 10. HLC, high fat diet-low fat diet control; HLR, high fat diet-low fat diet with resveratrol supplementation; HLE, high fat diet-low fat diet with exercise training. * $P < .05$ versus HLC.

Table 7. Comparison of IFN- γ production in each group

Group	HLC	HLR	HLE	F	p
LPS non Stimulated (pg/ml)	2.70 \pm 0.41	2.69 \pm 0.99	2.10 \pm 0.28	1.401	.284
LPS-stimulated (0.5 $\mu\text{g/ml}$) (pg/ml)	4.59 \pm 0.33	4.11 \pm 1.54	3.20 \pm 0.72	2.452	.128
LPS-stimulated (1.0 $\mu\text{g/ml}$) (pg/ml)	12.93 \pm 0.55	13.16 \pm 0.52	11.18 \pm 1.38* [†]	6.989	.010

Values are presented as mean \pm SD. n = 10. HLC, high fat diet-low fat diet control; HLR, high fat diet-low fat diet with resveratrol supplementation; HLE, high fat diet-low fat diet with exercise training. * $P < .05$ versus HLC. [†] $P < .05$ versus HLR.

Table 8. Comparison of IL-10 production in each group

Group	HLC	HLR	HLE	F	p
LPS non Stimulated (pg/ml)	86.56 \pm 14.11	84.52 \pm 10.41	84.52 \pm 9.86	.050	.952
LPS-stimulated (0.5 $\mu\text{g/ml}$) (pg/ml)	102.20 \pm 14.82	90.43 \pm 10.32	84.78 \pm 12.13	2.500	.124
LPS-stimulated (1.0 $\mu\text{g/ml}$) (pg/ml)	186.81 \pm 35.14	174.61 \pm 53.00	144.28 \pm 34.74	1.370	.291

Values are presented as mean \pm SD. n = 10. HLC, high fat diet-low fat diet control; HLR, high fat diet-low fat diet with resveratrol supplementation; HLE, high fat diet-low fat diet with exercise training.

with resveratrol intake and medium strength exercises on the obese mice, which were fattened with 8 weeks of high fat diet, IL-12p70 of peritoneal macrophages was similar with IL-6 that HLE group showed significant reduction compared to HLC group at the concentration of LPS-stimulation of 1.0 $\mu\text{g/ml}$ ($p = .003$, Table 6).

IFN- γ production

Upon the completion of 8 weeks of low fat diet combined with resveratrol intake and medium strength exercises on the obese mice, which were fattened with 8 weeks of high fat diet, IFN- γ expression level of peritoneal macrophages was similar with IL-6 and IL-12p70 and it was shown that HLE group had significant reduction compared to HLC group and HLR group ($p = .010$, Table 7) when LPS-stimulation was done at the concentration level of 1.0 $\mu\text{g/ml}$.

IL-10 production

Upon the completion of 8 weeks of low fat diet combined with resveratrol intake and medium strength exercises on the obese mice, which were fattened with 8 weeks of high fat diet, IL-10 expression level of peritoneal macrophages was not different among the groups (Table 8).

DISCUSSION

High fat diet and lack of exercise-related obesity causes chronic inflammation and thus brings problems such as cardiovascular disease, immune function decrease, etc.

Therefore, this study was aimed to review the effect of low fat diet combined with resveratrol administration and medium strength exercises on the obese mice, which were fattened with high fat diet, to study the weight and blood lipids and the expression level of proinflammatory cytokine from macrophage.

This study shows that medium strength exercises causes a significant reduction in body weight and total cholesterol and thus achieved the same result as with previous studies and it appears to be that medium strength exercises causes weight reduction due to energy consumption increase even when low fat diet was combined [19-21].

But resveratrol administration group had no difference of weight compared to the group which only had low fat diet.

Such result suggests that due to the effect of diet change from high fat to low fat in this study, resveratrol administration did not affect the weight reduction.

Although various studies reported blood lipid improvement, fat loss and anti-obesity effects of resveratrol, the result of this study and previous studies [15,22] which did not see the effect of resveratrol of various concentration on the obese model, which were fattened with high fat diet, suggest that weight reduction and blood lipid reduction can differ based on the level of obesity, the duration of resveratrol administration and its concentration and thus further studies are necessary in the future.

Low fat diet combined with resveratrol and medium strength exercises were analyzed upon the completion of non-LPS-stimulation and LPS-stimulation (0.5, 1.0 $\mu\text{g/ml}$) as the method of presenting antigen to measure proinflammatory cytokine, which was expressed from peritoneal macrophages, and the result showed that HLE group had significant decrease compared to HLC group for TNF- α and MCP-1 regardless of whether LPS-stimulation had been applied or not.

Such result was the same with the weight reduction and

according to previous studies [9,17] where expression of proinflammatory cytokine and weight are closely related and thus demonstrated that there is a correlation between weight reduction and expression decrease of inflammatory cytokine.

Leticia *et al.* [1] reported that expression level increase of proinflammatory cytokine such as TNF- α , IL-6 and IL-1 β in the obese mice fattened with high fat diet is the phenomenon caused by macrophage function decrease in obese mice and also regular exercise can improve the macrophage-mediated inflammation response.

And considering the decrease of inflammation caused by decreased fat synthesis in adipose tissue upon applying medium strength exercises after the completion of high fat diet [17], such proinflammatory cytokine is not only interrupting immune cells in the obese model but also expressed in excessive adipose tissues and with macrophage being infiltrated into adipose tissues, interfering with the regulation of various adipokine in adipose tissue [24].

And with such results, it was reported that excessive proinflammatory cytokine increased inflammation of plasma-level in circulation system and thus closely related to metabolic syndrome, hyperlipidemia, diabetes and hypertension [25].

Therefore, further studies on a correlation between macrophage infiltration and inflammation-level in adipose tissues in the obese model will be meaningful.

Medium strength exercises program (VO_2max 60-76%) in this study enhanced immune cell proliferation and also it was reported that DNA damage of lymphocyte circulating system received positive effect by not increasing epinephrine or norepinephrine [26].

It can be said that exercise intensity is an important factor effecting inflammation-level of the body considering the result of reduced proinflammatory cytokine despite of no weight reduction in obese mice which did voluntary wheel running exercise [27] and increased expression-level of proinflammatory cytokine with inflammatory response in the body by high intensity exercise or short-term exhaustive exercise even for the mice at normal weight range from previous studies [28,29].

This study had treated LPS with 2 levels of concentration (0.5, 1.0 $\mu\text{g/ml}$) as the antigen-presenting method of peritoneal macrophages and the result showed no difference among groups when LPS-stimulation was absent and when LPS-stimulation was applied with the concentration of 0.5 $\mu\text{g/ml}$ regarding the expression level of cytokines IL-6, IL-12p70 and IFN- γ , but when higher level of LPS-stimulation (1.0 $\mu\text{g/ml}$ concentration) was applied, HLE group was significantly decreased than HLC group.

In innate immunity, peritoneal macrophages react to

external stimuli such as LPS or bacteria or antigen by immune response.

Especially, IL-6 is known to have both of proinflammatory and anti-inflammatory characteristics and also closely related to the generation of TNF- α or CRP and IFN- γ is known to be involved with inflammatory cytokines synthesis.

This study showed that from the stimulation of exogenous antigen (LPS) at or more concentrated than 1.0 $\mu\text{g/ml}$, drastic increase of IL-6 and IFN- γ occurred and such result appears to be the active immune response of macrophage and in such case, it can be said that expression of inflammatory cytokines was significantly reduced in HLE group compared to HLC group and HLR group through the positive effect of medium strength exercises.

Regular exercise is known to control the inflammation-level in the body with anti-inflammatory activity [30] and reduces circulatory system CRP, adiponectin and IL-6 with inflammation-reduction mechanism and at the same time, affects the increase of anti-inflammatory cytokine such as IL-4 [31].

Expression level of IL-10 received no significant effect from medium strength exercises and resveratrol administration and it has been reported that IL-10 is getting affected by various factors including inflammation-level in the body and activity of T cells and B cells during the process of directly controlling inflammation response [32].

Therefore, further studies are required to determine whether anti-inflammation-level was effected by low fat diet of this study or the intensity and duration of exercise and the dose and duration of resveratrol has affected the expression of IL-10.

Recently Baur *et al.* [14] and other researchers [15] reported that resveratrol administration after high fat diet causes improved insulin sensitivity, triglycerides decrease, weight loss and fat reduction and the reduction of blood cholesterol [33] and thus it reported resveratrol as a beneficial polyphenol complex for obesity or anti-inflammatory activity.

In the obese model, one study reported that resveratrol administration (2 g or 4 g/day food) increased mitochondrial function and prevented metabolic diseases [13], and Baur *et al.* [14] reported that low concentration (20 mg/kg) of resveratrol administration increased life-span of obese mice regardless of weight reduction.

But on the other hand, one study [15] reported that administration of highly concentrated resveratrol (135 mg/kg, 282 mg/kg) for 15 weeks did not make a difference compared to the control group in terms of weight and adipose tissue and also Ingram *et al.* [34] reported that there was no diverse beneficial effects of resveratrol, which were reported from previous studies, when resveratrol was administered combined

with calorie restriction to the obese model.

And there was a study [35] which reported that resveratrol administration on the diabetic mice, which were induced from obesity, did not have effect on insulin resistance and Jun *et al.* [36] reported that when there was no obesity or having normal lipid metabolism or no resistance toward glucose, resveratrol administration did not affect on body composition or expression of inflammation in lipid metabolism and such result was caused by the fact that resveratrol administration did not affect the expression of SIRT1, UCP and AMPK in adipose tissue and muscle tissue and cell-signaling system and thus not effective for inflammation-reduction or metabolic disease-improvement and therefore there is no clear conclusion for the concentration or duration of resveratrol administration, whether the subject was obese or not and which diet pattern was given.

This study also showed the result that 8 weeks of resveratrol administration did not affect weight, blood lipids and expression of proinflammatory cytokine in macrophage.

Considering the results of previous studies, low fat diet in this study had stronger anti-obesity effect than resveratrol and thus the possibility of non-effect of resveratrol and also concentration levels for administration and duration might played some role and we think further studies are necessary due to our limitation of not administrating resveratrol on continuous-high fat diet or non-obese subjects.

CONCLUSION

This study found out the positive effect of medium strength exercises on weight and total cholesterol reduction by analyzing weight and blood lipids based on combined use of low fat diet and resveratrol and medium strength exercises and expression level of proinflammatory cytokine upon the completion of LPS-stimulation in macrophage in the mice which had been fed high fat diet.

And also the study found significant reduction of TNF- α and MCP-1 in the exercise-group regardless of LPS-stimulation when analyzed proinflammatory cytokine expressed in peritoneal macrophages and expression level of IL-6, IL-12p70 and IFN- γ was significantly lower in the exercise-group when LPS-stimulation was given at the concentration of 1.0 $\mu\text{g/ml}$.

But resveratrol administration did not show significant effect.

In conclusion, conducting medium strength exercises combined with low fat diet appears to be an ideal method for weight reduction or improving inflammation-level of

immune cells in the high fat diet model.

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