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Moderate Intensity Exercise Has More Positive Effects on The Gene Expression of Inflammasome, M1, M2 Macrophage Infiltration and Brown Adipocyte Markers Compared to High Intensity Exercise in Subcutaneous Adipose of Obese Mice Induced By High Fat Diet

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The purpose of the study was to compare the effect of either moderate or high intensity aerobic exercise on inflammasome, M1, M2 macrophage infiltration and brown adipocyte markers in subcutaneous adipose tissue of the high fat diet-induced obese mice. The 4 weeks male C57BL/6 mice were randomly assigned to four groups: normal diet control (NC; n=10), high-fat diet control (HC; n=10), high fat diet with moderate intensity exercise (HME; n=10), or high fat diet with high intensity exercise (HIE; n=10) groups. The high fat diet was given 60% calories from fat whereas normal diet was given 18% calories from fat. The moderate intensity exercise group (HME) was set at 10m/min in the first 2 weeks, 12m/min in 3-5 weeks and 14m/min in 6-16 weeks and the high intensity exercise group (HIE) was set at 14m/min in the first 2 weeks, 17m/min in 3-5 weeks and 18m/min in 6-16 weeks. The semi quantitative reverse transcription-polymerase chain reaction (RT PCR) was used to analyze the gene expression. The moderate intensity exercise significantly reduced the expression of NLRP3, F480, CD11c and CD86. Further, the moderate intensity exercise significantly increased CD206 and PGC1α, BMP7 and PRDM. The high intensity exercise significantly reduced NLRP3, CD11c and CD86. Further, the high intensity exercise significantly increased PGC1α and BMP7. In conclusion, moderate intensity exercise has more positive effects on inflammasome, M1, M2 macrophage infiltration and brown adipocyte maskers compared to high intensity exercise in high fat diet induced obese mice.

Key words : Brown adipocyte, inflammasome, intensity exercise, macrophage infiltration, obese

Introduction

Obesity is a serious health problem that is dramatically increase and epidemic in most countries [21]. Obesity is characterized by conditions of low-level chronic inflammation with infiltration of immune cells into adipose tissue progressively [1]. The obesity causes inflammatory changes in white adipose tissue (WAT), characterized by the irregular expression of inflammatory adipokines involving tumor necrosis factor-α (TNF-α) and mono-chemoattractant protein-1 (MCP-1), and contributes to metabolic complication [14, 16]. Other study has found that there is a strong correlation between obesity, inflammation and NLRP3 inflammasome expression in abdominal subcutaneous adipose tissue.

Knockout NLRP3 inflammasome in mice has been reported to protect from obesity-associated macrophage activation in adipose tissue, reducing the M1-like macrophage activation in adipose tissue and increasing the expression of M2-like cytokine [4]. Macrophages in adipose tissue play an important role in the formation of chronic inflammatory conditions and metabolic dysfunction [1]. Obese-induced white adipose tissue dysfunction further can lead to metabolic complications. White adipose tissue plays a role in energy storage, whereas brown adipose tissue specifically for thermogenic energy expenditure [5].

The main etiological factors for obesity are nutritional overload and lack of physical activity, indicating that obesity is lifestyle-dependent and can be prevented. Exercise is an important lifestyle factor widely used as a tool to prevent and improve lifestyle-related obesity and insulin resist-

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ance [9]. Exercise is useful for increasing energy expenditure to prevent a positive energy balance and reducing the activation of inflammation and oxidative stress pathway. Physical activity has long been known to decrease cell size and lipid level, and increase mitochondrial biogenesis in WAT [19]. Moderate exercise training has beneficial effect on body weight, total cholesterol, peritoneal macrophage infiltration and pro-inflammatory cytokine production in high fat diet mice. In rodent models, exercise training increases mitochondrial biogenesis and activity in adipose tissue [18]. Exercise training also increases expression of the brown adipocyte marker uncoupling protein 1 (UCP1) in adipose tissue. Although these effects are much more pronounced in subcutaneous adipose tissue. Consistent with the increase in UCP1, exercise training increases the presence of brown-like adipocytes in subcutaneous white adipose tissue [17, 20]. Exercise decreases those circulating levels of lipopolysaccharide (LPS) and TLR 4 activity along with TNF-α, IL-1-β and MCP-1 mRNA levels in white adipose tissue [12].

Our previous study found that moderate exercise training with low fat diet improves lipid metabolism, mitochondrial biogenesis and inflammation process. Further it inhibits macrophage infiltration and promotes M2 macrophage markers in the skeletal muscle and adipose tissue in high fat diet induced obese mice [6, 7, 10].

Taken together, moderate intensity exercise has good effects and being extensively researched against obesity and its complications. However, the effects of high intensity exercise have not been investigated widely on obese induced metabolic complication. Further, the effects of both moderate and high intensity exercise have not been analyzed on inflammasome, inflammation, macrophage infiltration and browning markers. Therefore, in this study, we test to compare the effects of moderate and high intensity on the high fat diet-induced obese metabolic complication.

Materials and Methods

Animal and diet

Male C57BL/6 mice 4 weeks old, n=40 from Central Experimental Animal, Korea were housed in cages (5 mice per cage) with standard experimental laboratory, at temperature 22±2˚C, with 60±5% humidity. After one-week adaptation period, the mice were feed either a high fat diet (60% of calories from fat, 20% from carbohydrate, 20% from protein, Orient Bio Inc., #D12492) or a normal diet (18% calories from fat, 58% from carbohydrate, 24% from protein, Orient Bio Inc., #2018) ad libitum for 15 weeks. The mice were randomly assigned to four groups: normal diet control (NC; n=10), high fat diet control (HC; n=10), high fat diet with moderate exercise training (HME; n=10), and high fat diet with high intensity exercise training (HIE; n=10) groups. The mice were weighed and food intake per cage (5 mice) measured weekly. All experiments were approved by the Animal Care and Use Committee at the Chungnam National University (CNU-00494).

Exercise Program

Exercise training will be initiated from the first week until the sixteenth week that consists of two type intensity: moderate and high-intensity exercise. For moderate exercise group (HME) the running speed was set at 10 m/min in the first 2 weeks, 12 m/min in 3-5 weeks and 14 m/min in 6-16 weeks. For high-intensity exercise group (HIE) the running speed was set at 14 m/min in the first 2 weeks, 17 m/min in 3-5 weeks and 18 m/min 6-16 weeks. The exercise training mice placed on a treadmill for 40-60 min/day, 4 days/week. During exercise, the control group also exposed the same environmental stresses from treadmill noise and vibration. In order to minimize stress during exercise, external stimulation or electrical shock not allowed [14].

All the mice were sacrificed after fasted for 12 hr under anesthesia using a mixture ketamine (80 mg/kg) and xylazine (10 mg/kg). The subcutaneous adipose tissue was quickly removed, weighed and frozen in liquid nitrogen and stored at -80˚C until analysis.

RNA extraction and semi quantitative reverse transcription-polymerase chain reaction (RT PCR)

Total RNA was extracted from 150 mg subcutaneous adipose tissue homogenate using 1 ml Trizol reagent (Ambion, Carlsbad, CA, USA). The total RNA concentration was calculated by measuring the absorbance at 260 nm and 280 nm using an ultraviolet spectrophotometer. For cDNA synthesis, Maxi RT PreMix kit (iNtRON, Korea) was used according to the manufacturer's instructions.

The PCR was set using the following program: 95°C for 2 min, 95°C for 30 sec, 38-40 cycle, the appropriate annealing temperature between 55-57°C for 30 sec and 72°C for 2 min. After loaded onto 1% of the agarose gel containing ethidium bromide then measured the PCR band density with Image

Lab 4.0 (Bio-Rad, USA). The mRNA levels were normalized with β-Actin. The PCR primer sequences for each studied gene are shown in Table 1.

Statistical Analysis

Statistical analysis from RT-PCR data was performed by SPSS V22.0 using one-way ANOVA with LSD post-hoc tests. Statistical significance was defined as α=0.05.

Results

Fig. 1. Shows the changes of Calorie intake, body weight and subcutaneous adipose tissue. In Calorie intake there is significant increase in HC group compare to NC group. HIE group have significant effect in calorie intake compared to HC group.

The body weight significantly increases in all group compared to NC group. The subcutaneous adipose tissue weight significant increase in HC group and significantly decrease in HME group.

Inflammasome marker

Fig. 2 showed the effect of high fat diet on inflammasome marker with moderate intensity exercise and high intensity exercise. There is a significant increase in HC group compared to NC group in NLRP3 mRNA expression.

Macrophage Marker

There is a significant increase in all M1 macrophage marker mRNA expression in HC group compared with NC group. Moderate exercise can decrease the M1 macrophage marker CD11c and CD86. M2 macrophage marker CD206 significant increase by moderate exercise.

Browning Marker

PGC1α, BMP, UCP1, PRDM and PPARα mRNA expression significant decrease in HC group. Moderate exercise significant increase PGC1α, BMP, PRDM and PPARα mRNA expression compared to HC group. PGC1α mRNA expression significant increase in HIE group compared to HC group.

Discussion

In this study, the body weight and calorie intake were not significantly changed by moderate and high intensity exercise. This results are similar with other research that found exercise does not affect weight loss in groups with high fat diets nor does it affect some inflammation markers such as TNF-alpha, MCP1 and Leptin. Beside that macrophage infiltration marker F4/80 has decreased slightly with exercise [2].

The reductions in inflammatory gene expression in high fat diet with exercise were likely the result of decreased epididymal fat pad size and reductions in gene expression of MCP-1, F4/80, and the M1 macrophage polarization marker, CD11c [17]. We found that there is significant decreasing size of subcutaneous adipose tissue weight in moderate exercise group but not accompanied with inflammatory cytokine that not change in the group high fat diet

Table 1. Reverse transcription-polymerase chain reaction primer sequences

Gene	Forward	Reverse
NLRP3	GCTCCAACCATTCTCTGAC	AGTTACACTGTGGGTCCTT
ASC	CAGGTATTGCCATCATTCAGT	TTCCATAGGTAGGACCATAAATAA
Caspase 1	GCAAAGAGGAAGCAATTTATCA	GCCTTGTCCATAGCAGTAAT
IL-1β	TCACAAGCAGAGCACAAG	GAAACAGTGCAGCCCATAC
IL-18	AATCTGTAATGTTCACTCTCACTA	GCCTCGGGTATTCTGTTATG
F480	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
CD11c	GAGAGCCCAGACGAAGAC	TTTGAAGAAACCAGCCTTGTAA
CD86	TCTCCACGGAAACAGCATCT	CTTACGGAAGCACCCATGAT
CD206	CAGGTGTGGGCTCAGGTAGT	TGTGGTGAGCTGAAAGGTGA
PGC-1α	GCTCCAACCATTCTCTGAC	AGTTACACTGTGGGTCCTT
BMP	CAGGTATTGCCATCATTCAGT	TTCCATAGGTAGGACCATAAATAA
UCP1	GCAAAGAGGAAGCAATTTATCA	GCCTTGTCCATAGCAGTAAT
PRDM	TCACAAGCAGAGCACAAG	GAAACAGTGCAGCCCATAC
PPARα	AGGCAGATGACCTGGAAAGT	CTCCTCACCGATGGACTGA
β-actin	TCACCCACACTGTGCCCATCACGA	CAGCGGAACCGCTCATTGCCAATGG

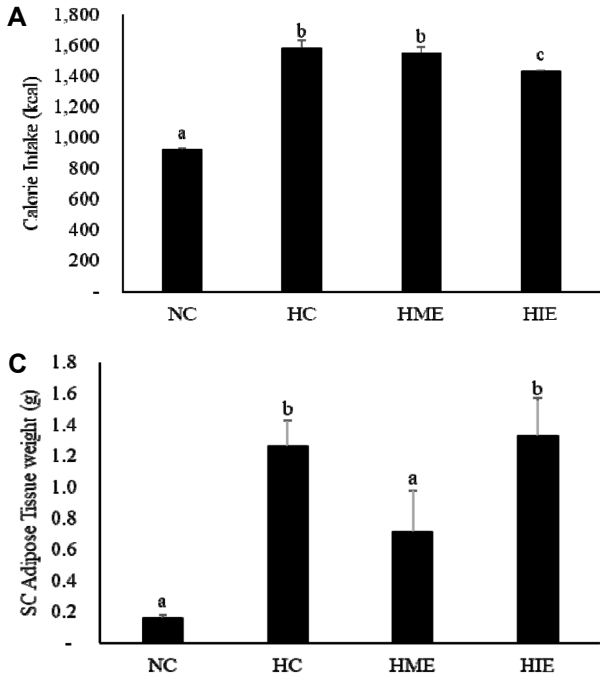

Fig. 1. The change of (A) Body Weight; (B) Subcutaneous Adipose Tissue (SAT) weight of high-fat- diet-induced obese mice. Values represent means ± SEM. Different alphabet means significant difference.

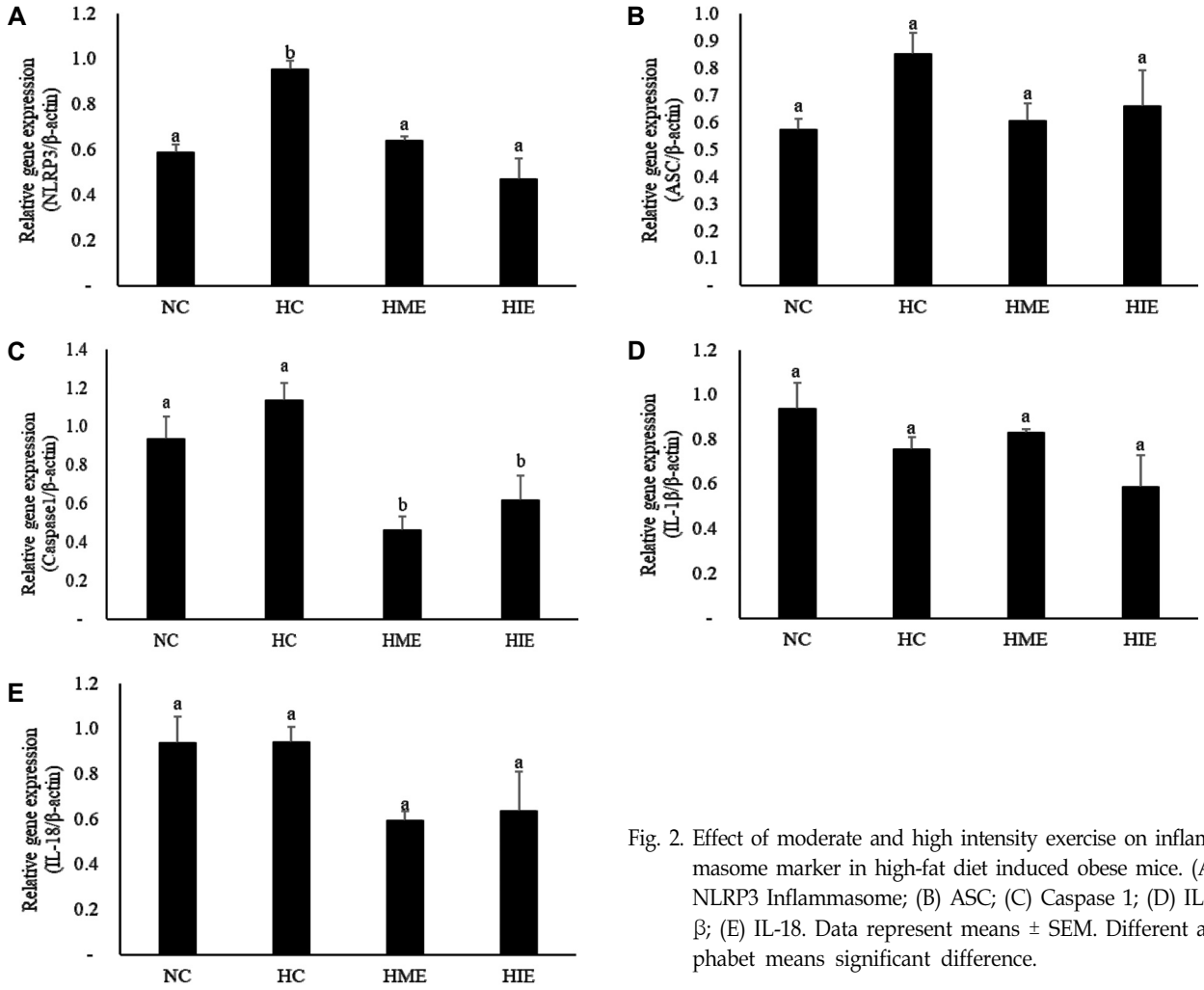

Fig. 2. Effect of moderate and high intensity exercise on inflammasome marker in high-fat diet induced obese mice. (A) NLRP3 Inflammasome; (B) ASC; (C) Caspase 1; (D) IL-1 β; (E) IL-18. Data represent means ± SEM. Different alphabet means significant difference.

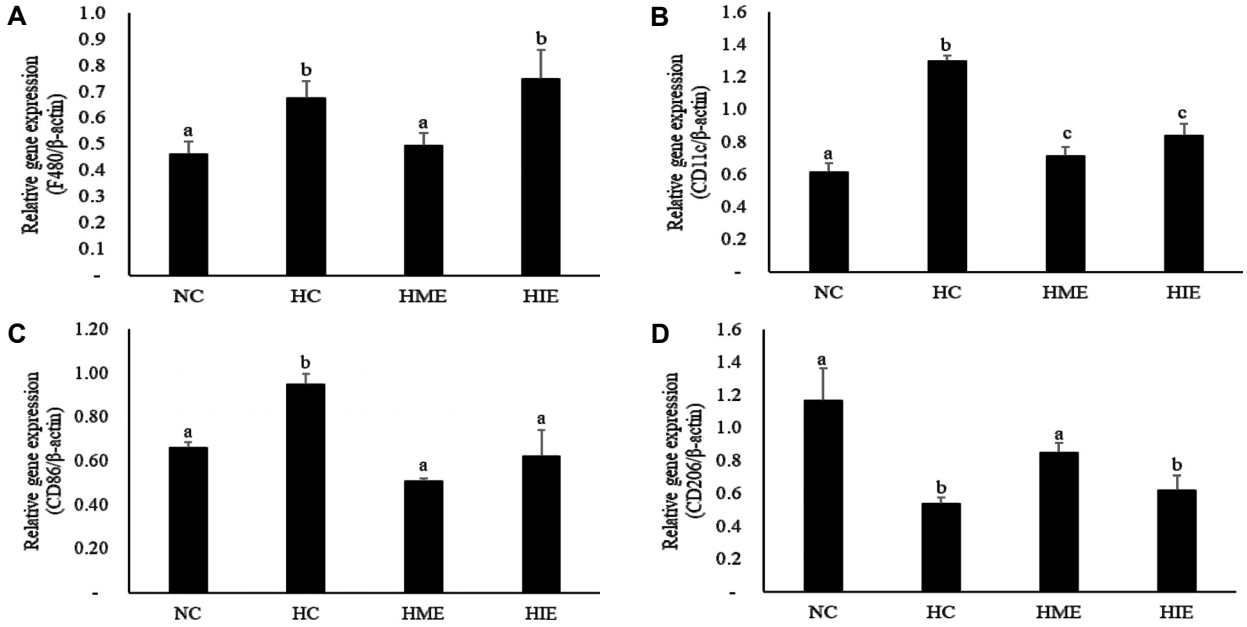

Fig. 3. The effect of moderate and high intensity exercise on macrophage marker in high-fat diet induced obese mice. (A) F480, (B) M1 macrophage marker CD11c, (C) M1 macrophage marker CD86, (D) M2 macrophage marker, CD206. Data represent means ± SEM. Different alphabet means significant difference.

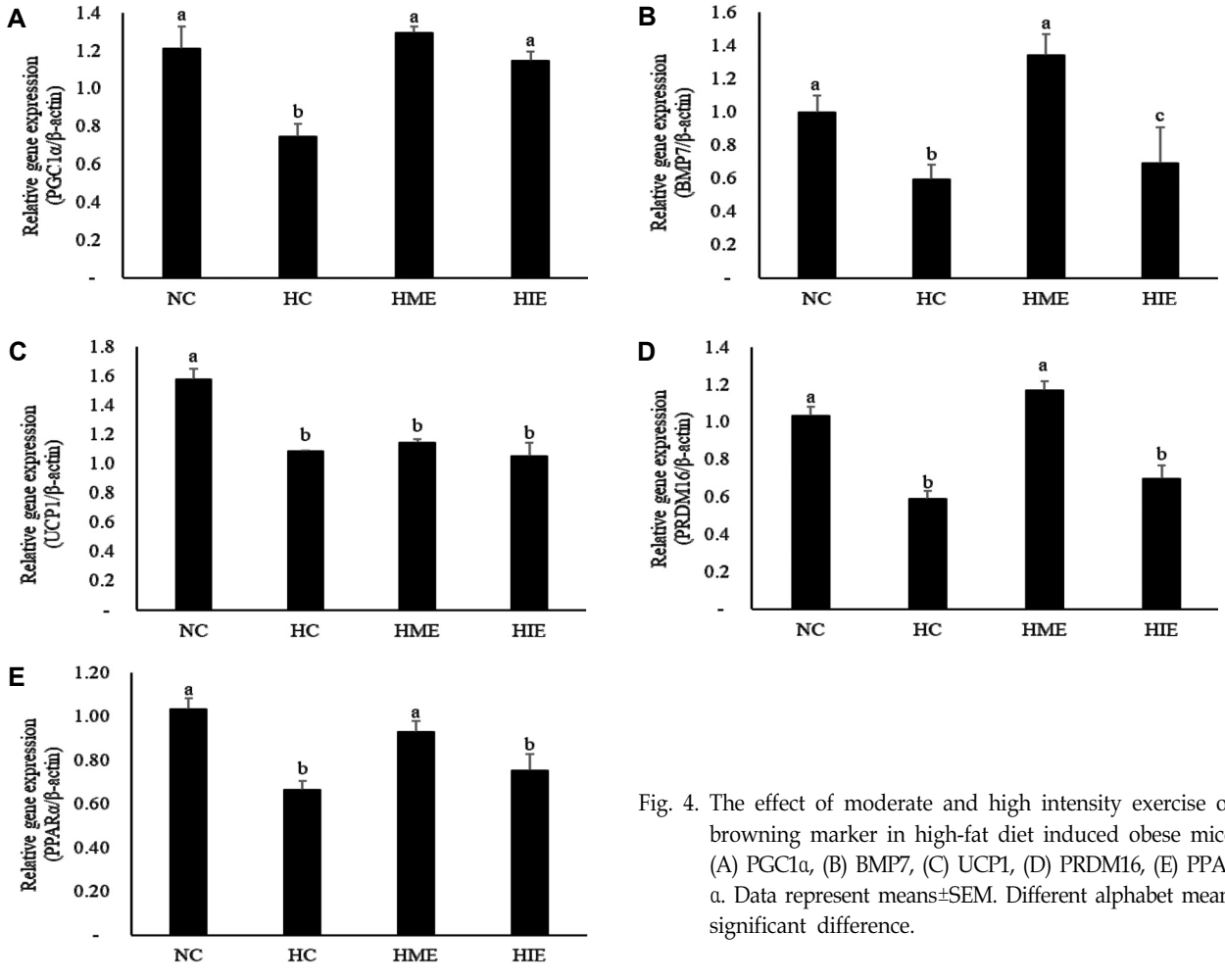

Fig. 4. The effect of moderate and high intensity exercise on browning marker in high-fat diet induced obese mice. (A) PGC1α, (B) BMP7, (C) UCP1, (D) PRDM16, (E) PPAR α. Data represent means±SEM. Different alphabet means significant difference.

with moderate intensity exercise or high intensity exercise. Several small randomized trials utilizing high-intensity exercise, characterized by brief intermittent bouts of high-intensity aerobic exercise have emerged over recent years, and revealed impressive effects on pro-inflammatory production relative to moderate intensity continuous training [15]. The immune response using serum cytokines indicated that high-intensity exercise performed by highly trained obese rodent only generated inflammation that was localized to the skeletal muscle [3].

The previous study found that NLRP3 inflammasome plays a substantial role in sensing obesity‐associated inducers of caspase-1 activation and therefore regulates the magnitude of the inflammation [6]. Recent study found that there was significant increase of NLRP3 inflammasome but not in ASC and inflammatory cytokine IL1β and IL-18 in high fat diet group compared to normal diet group. However moderate intensity exercise and high intensity exercise has significantly reduced the mRNA expression of NLRP3 inflammasome. These finding suggest that exercise has modest benefits on NLRP3 inflammasome regulation in the high fat diet induced obese mice.

We also did research on M1 and M2 macrophage infiltration marker and found that moderate intensity exercise has significant effect on F480, CD11c, CD86 and CD206 weather high intensity exercise has significant effect only on CD11c and CD86 as M1 macrophage marker. It is evident that moderate intensity exercise with treadmill training have protective effect on white adipose tissue macrophage activation and inflammation [2, 8, 17]. These findings have been supported in other study that mentioned moderate exercise training induced macrophage phenotypic switching from M1 to M2 in adipose tissue [8].

Our previous study has found that aerobic exercise with low fat diet has a positive effect to immune system in obese mice [10, 16]. Another our previous study mentioned that exercise training has beneficial effects on body weight, total cholesterol, peritoneal macrophage and pro-inflammatory cytokine in high fat diet mice [13].

The exercise can make a process browning on white adipose tissue. We found that high fat diet with moderate exercise can induced browning marker in subcutaneous adipose tissue. 16 wk of exercise training with calorie restriction does not result in increased UCP1 expression or of any other bright biomarker despite evidence for the molecular remodeling of adipose tissue [11].

BMP7-induced UCP1 expression was markedly diminished in brown pre-adipocytes deficient in both PGC-1α and PGC-1β. In this regard, a new role for BMP7 in brown adipogenesis and consequent increased energy expenditure has been suggested. Altogether, data suggest that BMP7 activates a full program of brown adipogenesis including the induction of early regulators of brown fat fate PRDM16 and PGC-1α, increased expression of the brown-specific marker UCP1 and adipogenic transcription factors PPARɣ and CCAAT/enhancer-binding proteins, and induction of mitochondrial biogenesis via p38 mitogen-activated protein kinase and PGC-1α-dependent pathways [19-22].

Exercise increased the transcript levels of Bmp7 gene and others brown adipocyte-specific markers (Cidea, PRDM16 and UCP1), but did not modulate the native beige adipocyte-specific marker Tmem26 [14].

In conclusion moderate intensity exercise has positive effect on inflammasome, macrophage marker, and browning marker in high fat diet induced obese mice. However high intensity exercise has less effect on that.

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References

1. Bai, Y. and Sun, Q. Macrophage recruitment in obese adipose tissue. 2015. *Obes. Rev.* **16**, 127-136.
2. Baynard, T., Vieira-Potter, V. J., Valentine, R. J. and Woods, J. A. 2012. Exercise training effects on inflammatory gene expression in white adipose tissue of young mice. *Mediators Inflamm.* **28**, 202-210.
3. Bessa, A., Oliveira, V. and Espindola, F. 2016. Exercise intensity and recovery: Biomarkers of injury, inflammation, and oxidative stress. *J. Strength. Con. Res.* **30**, 311-319.
4. Harms, M. and Seale, P. 2013. Brown and beige fat: development, function and therapeutic potential. *Nat. Med.* **19**, 1252-1263.
5. Jeong, J. H., Park, H. G., Lee, Y. R. and Lee, W. L. 2015a. Moderate exercise training is more effective than resveratrol supplementation for ameliorating lipid metabolic complication in skeletal muscle of high fat diet-induced obese mice. *J. Exerc. Nutr. Biochem.* **19**, 131-137.
6. Jeong, J. H., Lee, Y. R., Park H, G. and Lee, W. L. 2015b. The effects of either resveratrol or exercise on macrophage infiltration and switching from M1 to M2 in high fat diet mice. *Obesity* **19**, 65-72.

7. Kawanishi, N., Yano, H., Yokogawa, Y. and Suzuki, K. Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. *Exerc. Immunol. Rev.* **16**, 105-118.
8. Krüger, K., Mooren, F. C. and Eder, K. 2016. Immune and Inflammatory Signaling Pathways in Exercise and Obesity. *Am. J. Lifestyle Med.* **10**, 268-279.
9. Kwon, S. M., Park, H. G., Jun, J. K. and Lee, W. L. 2014. Exercise, but not quercetin, ameliorates inflammation, mitochondrial biogenesis, and lipid metabolism in skeletal muscle after strenuous exercise by high-fat diet mice. *J. Exerc. Nutr. Biochem.* **18**, 51-60.
10. Linden, M. A., Pincu, Y., Martin, S. A., Woods, J. A. and Baynard, T. 2014. Moderate exercise training provides modest protection against adipose tissue inflammatory gene expression in response to high-fat feeding. *Physiol. Rep.* **2**, 1-13.
11. Nakhuda, A., Josse, A. R., Gburcik, V., Crossland, H., Raymond, F., Metairon, S., Good, L., Atherton, P. J., Phillips, S. M. and Timmons, J. A. 2016. Biomarkers of browning of white adipose tissue and their regulation. *Am. J. Clin. Nutr.* **104**, 557-565.
12. Oliveira, A. G., Araujo, T. G., Carvalho, B. M., Guadagnini, D., Rocha, G. Z., Bagarolli, R. A., Carvalheira, J. B. C. and Saad, M. J. A. 2013. Acute exercise induces a phenotypic switch in adipose tissue macrophage polarization in diet-induced obese rats. *Obesity* **21**, 2545-2556.
13. Park, H. G., Lee, Y. R., Jun, J. K. and Lee, W. L. 2014. Exercise training is more effective than resveratrol supplementation on alleviation of inflammation in peritoneal macrophages of high fat diet mice. *J. Life Sci.* **18**, 79-87.
14. Park, J. G., Kim, Y. A., Park, H. G. and Lee, W. L. 2018. Aerobic exercise amelirorated high fat diet induced endoplamic reticulum stress more than polyphenol supplementation in skeletal muscle of obese mice. *J. Life Sci.* **28**, 1186-1192.
15. Ramos, J. S. and Dalleck, L. C. 2015. The impact of high-intensity interval training versus moderate intesity continuous triaining on vascular function: a systematic review and meta-analysis. *Sports Med.* **45**, 679-692.
16. Rocha-Rodrigues, S., Rodríguez, A., Gouveia, A. M., Gonçalves, I. O., Becerril S., Ramírez, B., Beleza, J., Frühbeck, G., Ascensão, A. and Magalhães, J. 2016. Effects of physical exercise on myokines expression and brown adipose-like phenotype modulation in rats fed a high-fat diet. *Life Sci.* **165**, 100-108.
17. Sakurai, T., Ogasawara, J., Shirato, K., Izawa, T., Oh-Ishi, S., Ishibashi, Y., Radák, Z., Ohno, H. and Kizaki, T. 2017. Exercise training attenuates the dysregulated expression of adipokines and oxidative stress in white adipose tissue. *Oxid. Med. Cell. Longev.* **4**, 25-31.
18. Schulz, T. J. and Tseng, Y. H. 2013. Brown adipose tissue: development, metabolism and beyond. *Biochem. J.* **453**, 167-178.
19. Stanford, K. I., Middelbeek, R. J. W. and Goodyear, L. J. 2015. Exercise effects on white adipose tissue: Beiging and metabolic adaptations. *Diabetes* **64**, 2361-2368.
20. Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N. and Kahn, C. R. 2008. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* **454**, 1000-1004.
21. World Health Organization, "Obesity and Overweight: Fact Sheet," 2016. [Online]. Available: http://www.who.int/mediacentre/factsheets/fs311/en/. [Accessed: 20-May-2017].
22. Vandanmagsar, B., Youm, Y. and Ravussin, A. 2011. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat. Med.* **7**, 15-24.

초록 : 비만모델에서 중강도 운동에 의한 인플라마좀, 대식세포 침윤, 갈색지방 관련 바이오 마커의 개선 효과

김용안[1] · 피핏 피트리아니[2] · 박희근[1] · 이왕록[1*]
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비만은 체내 과도한 지방 축적으로 인하여 지방세포 자체에서 염증성 사이토카인이 증가로 인하여 세포의 기능을 약화시킨다. 규칙적인 운동은 지방분해와 갈색지방 증가로 인해 비만의 치료방법 중 핵심 전략으로 적용되고 있다. 고강도 운동은 염증성 사이토카인과 근형질세망 스트레스 발생을 초래하여 지방대사에 부정적인 결과를 초래하는 것으로 알려져 있다. 그러나 비만모델에서 중강도 운동과 고강도 운동에 의한 인플라마좀, 대식세포 침윤, 갈색지방 관련 바이오 마커의 비교연구는 이루어진 바 없다. 따라서 이 연구의 목적은 중강도 유산소 운동과 고강도 운동을 비교하여 인플라마좀(NLRP3, ASC), 대식세포 침윤인자 M1 (CD11c, CD86), M2 (CD206), 갈색지방(PGC1α, BMP7, PRDM, UCP1) 관련 변인에 우선적인 효과가 있는지 비교분석하고자 하였다. 이 연구의 목적을 위해 1) 정상식이 그룹(normal diet control, NC; n=10), 2) 60% 고지방식이 그룹(high-fat diet control, HC; n=10), 3) 중강도 운동 그룹(high fat diet with moderate intensity exercise, HME; n=10), 4) 고강도 운동그룹(high fat diet with high intensity exercise, HIE; n=10)으로 나누어 실시하였다. 중강도 운동 그룹은 고지방식이 그룹과 비교하여 NLRP3, F480, CD11c, CD8의 발현이 유의하게 낮아졌다. 중강도 운동은 CD206, PGC1α, BMP7, PRDM이 유의하게 증가하였다. 고강도 운동은 NLRP3, CD11c and CD86은 유의하게 감소한것으로 확인되었다. 그러나 고강도 운동은 PGC1α, BMP7는 증가한다. 이러한 결과는 중강도 운동은 인플라마좀, 대식세포 M1, M2 침윤과 갈색지방 세포 관련 요인의 개선이 효과적인 것으로 나타났다.