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Moderate Intensity Exercise Eliminates the Inflammation Marker in the Liver of High-Fat-Diet-Induced Obese Mice

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***Abstract*—The obese metabolic complication has already occurred nowadays. The purpose of the study was to investigate the effect of moderate intensity exercise on inflammation marker in the liver of high fat-diet-induced-obese mice. The 4 weeks male C57BL/6 mice were randomly dispersed to three groups: normal diet control (NC; n=10), high-fat diet control (HC; n=10), a high fat diet with moderate intensity exercise (HME; 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. The inflammation markers were checked by quantitative Real Time PCR. The body and liver weight was significantly increased in the high fat diet group. The moderate intensity exercise significantly reduced the gene expression of TNFα, and IL-1β the pro inflammatory cytokine IL-10 was significantly decreased in the high fat diet group but not with moderate intensity exercise. In conclusion, moderate intensity exercise has a positive effect on the liver of high-fat-diet-induced obese by reducing inflammation gene expressions. However, there was no effect of moderate intensity exercise on TLR4 and IL-10.**

Keywords—exercise; inflammation marker; obesity

I. INTRODUCTION

The obese metabolic complication has already occurred nowadays. The obesity-associated state of chronic low-grade systemic inflammation is a proinflammatory condition in which hypertrophied adipocytes and adipose tissue-resident immune cells, both contribute to increased circulating levels of proinflammatory cytokines [1,2]. This proses not only occur in adipose tissue but also in a liver cell that promotes the non-alcoholic fatty liver disease [3].

The fatty liver was formed by accretion of hepatic lipid from enhanced free fatty acids (FFA) influx and de novo lipogenesis [4–7]. The FFA generates oxygen free radicals (OFR) in the mitochondrial chain that causing lipid peroxidation and induces pro-inflammatory cytokine synthesis due to Kupffer cells and hepatocytes, such as tumor necrosis factor-alpha (TNF-α), transforming growth factor beta-1 (TGF-β1), FAS ligand, and interleukin-8 (IL-8) [7].

Exercise increases energy expenditure and burns off some of the body fat that would otherwise accumulate in individuals who consume more dietary energy than they need, therefore, exercise reduces the risk of developing obesity and excessive adiposity [8]. Current evidence supports that exercise training, such as aerobic and resistance exercise, reduces chronic inflammation, especially in obese individuals with high levels of inflammatory biomarkers undergoing a longer-term intervention [9,10].

Peripheral adipose tissue and hepatic insulin resistance were diminished in weight loss whereas regular exercise changes the development of fatty liver disease [4,11,12]. Aerobic-exercise-training ameliorates liver fat accumulation in mice without instigating any changes in adipose tissue mass [11,13]. Furthermore, the hepatic TNF-α, F4/80, chemokine expression and hepatic fibrosis markers in the liver attenuate by exercise training [13].

Since exercise has a good effect on inflammation in obesity especially adipose tissue, it will be good understanding the effect of exercise in the liver. Therefore, the purpose of this study is to investigate the effects of moderate intensity exercise on inflammation marker in the liver of high fat diet induced obese mice.

II. METHOD

This experimental study using four weeks old male C57BL/6 mice, n=60 were bought from the Central Experimental Animal, Korea. After one-week adaptation period, the mice fed by 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 16 weeks. They were caged by 5-mice-per-cage with standard experimental laboratory, at temperature 22±2˚C, and 60±5% humidity. The mice weighed the body weight and food intake per cage (5 mice) every week. The calorie intake is calculated from the total food intake conversion to calories. The mice were randomly assigned to 3 groups: normal diet control (NC; n=10), high-fat diet control

(HC; n=10) and e high-fat diet with moderate intensity exercise (HME; n=10).

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

All the mice were sacrificed after fasted for 12 hours under anesthesia using a mixture of ketamine (80mg/kg) and xylazine (10mg/kg). Before analysis, the tissues of liver were removed, weighed, frozen in liquid nitrogen, and stored at -70˚C.

Total RNA was extracted from 50gr liver tissue homogenate using 1ml 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. Polymerase Chain Reaction (PCR) was performed with the CFX 96 touch real-time PCR detection system using the Smobio ExcelTaq 2X Q-PCR Master Mixed. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as the housekeeping gene, and all data represented relative to its expression.

Statistical analysis was performed by SPSS V22.0 using One-way ANOVA with LSD posthoc tests. Statistical significance was defined as α=0.05.

III. Results and Discussion

The study result of calorie intake was shown in Figure 1. The calorie intake was significantly increased in high-fat diet control group with respect to normal diet control group. However, the high-fat diet with moderate intensity exercise group was not significantly different with respect to the high-fat diet control group.

High-fat diets used to induce obese contain 32% to 60% of calories from fat. Other study reported that diets with 60 kcal% fat are frequently used to induce obesity in rodents [14,15]. There are some mice carry a genetic trait that predisposes them to store fat when dietary fat content is high such as the C57BL/6 mice [16].

In a recent study, we used a normal diet with 18% of calories from fat and high-fat diet with 60% of calories from fat for 15 weeks. A recent study found there was not significantly different in the high fat diet with moderate intensity group. This founding is contrary with the previous study that mentioned increasing exercise intensity leads to an increase in calorie intake during the meal that follows the exercise session [17]. The differences in methods among these studies, particularly exercise intensity, nutritional status, gender, macronutrient composition of test foods and time intervals between exercise and eating affect these inconsistencies [18,19].

The study result in body weight was shown in Figure 2. We found that the body weight of C57BL/6 mice was significantly increased in the high-fat diet control group. Exercise is able to change energy balance in short to medium period however in the absence of energy restriction, long-term exercise affects the only a small amount of weight loss [20–25]. Moderate-intensity exercise without weight loss or calorie restriction is associated with substantial reductions in total and visceral fat, and skeletal muscle lipid with a significant improvement in fitness levels in obese individuals [26]. Some physiological and metabolic process may explain the lack of exercise effectiveness as a weight-loss strategy, first, exercise affects changes in body composition by reducing fat mass but maintaining fat-free mass, second, exercise is related with a decrease in energy expended in basal metabolic rate as a result of weight loss [24].

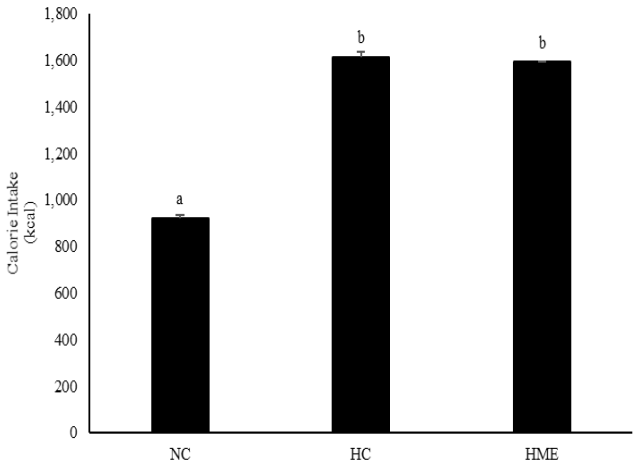

Fig. 1. Effect of moderate intensity exercise on the calorie intake of high fat diet induced obese mice. Data represent means±SE. Different alphabet means significant difference.

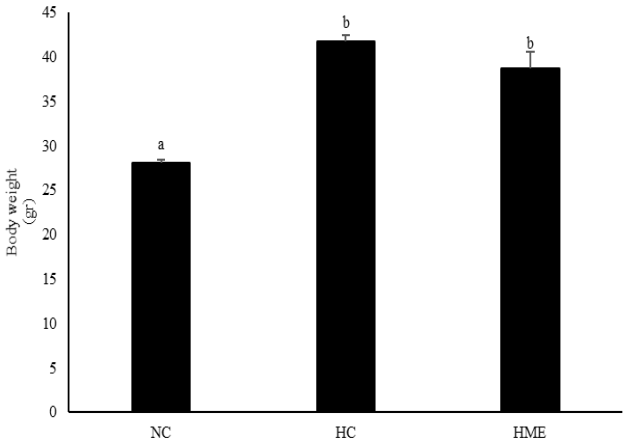

Fig. 2. Effect of moderate intensity exercise on body weight of high fat diet induced obese mice. Data represent means±SE. Different alphabet means significant difference.

The study result in liver weight was shown in Figure 3. The liver weight of the high-fat diet control group was significantly increased with respect to the normal diet control group (p=0.00). However, the liver weight of a high-fat diet with moderate-intensity exercise groups was not significantly different with respect to the high-fat diet control group.

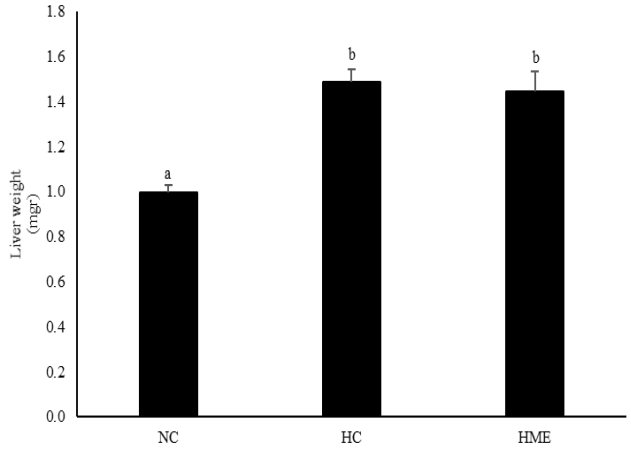

Fig. 3. Effect of moderate intensity exercise on the liver weight of high fat diet induced obese mice. Data represent means±SE. Different alphabet means significant difference.

Application of lifestyle interventions, such as caloric restriction and exercise, is highly recommended for the treatment of obesity and associated metabolic dysfunction [9]. The anti-inflammatory effects of exercise have been established [8,10,27]. High fat diet induced obesity metabolic inflammation in the adipose tissue leads to inflammation in the liver [28]. Tumor necrosis factor-α (TNFα) is an inflammatory cytokine involved in liver inflammation and sustained liver inflammation leads to liver fibrosis. TNFα exerts inflammation, proliferation, and apoptosis [29].

The study result of TNFα gene expression was shown in Figure 4. The TNFα gene expression of high-fat diet control was significantly increased with respect to the normal diet control group ($p<0.01$). The gene expression of TNFα was significantly decreased in the high-fat diet with moderate intensity exercise ($p<0.05$) with respect to the high-fat diet control group. This result was similar to our previous study that mentioned moderate intensity exercise ameliorate the TNF-α [30–32]. Moderate intensity exercise decreases TNF-α mRNA manifestation in adipose tissue and liver obese mice [33,34]. TNF-α have a special character in the growth of NASH by alluring hepatic fibrosis and injury [35]. Therefore, moderate exercise training may overturn TNF-α, which is a specific marker for NASH due to high fat diet-induced obesity.

The study result of IL-1β gene expression was shown in Figure5. The IL-1β gene expression of high-fat diet control was significantly increased with respect to the normal diet control group (p=0.00). The gene expression of IL-1β was significantly decreased in the high-fat diet with moderate intensity exercise group (p=0.00) with respect to the high-fat diet control group.

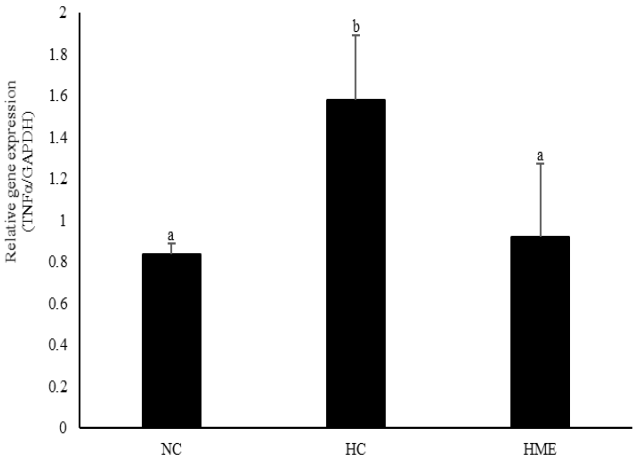

Fig. 4. Effect of moderate intensity exercise on inflammation marker TNFα in high fat diet induced obese mice. Data represent means±SE. Different alphabet means significant difference.

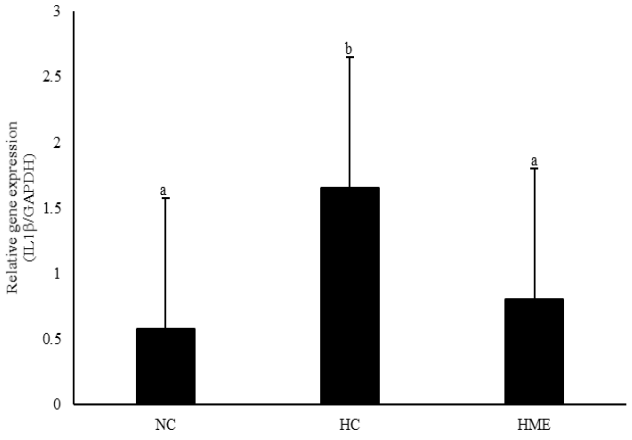

Fig. 5. Effect of moderate intensity exercise on IL1β in high fat diet induced obese mice. Data represent means±SE. Different alphabet means significant difference.

The study result of TLR4 gene expression was shown in Figure 6. The TLR4 gene expression was not significantly increased in high-fat diet control group with respect to normal diet control group (p=0.794). However, the high-fat diet with moderate intensity exercise was significantly reduced the TLR4 gene expression with respect to the high-fat diet control group. Regular exercise acts as anti-inflammatory agent by regulating immune TLR4 cell [36].

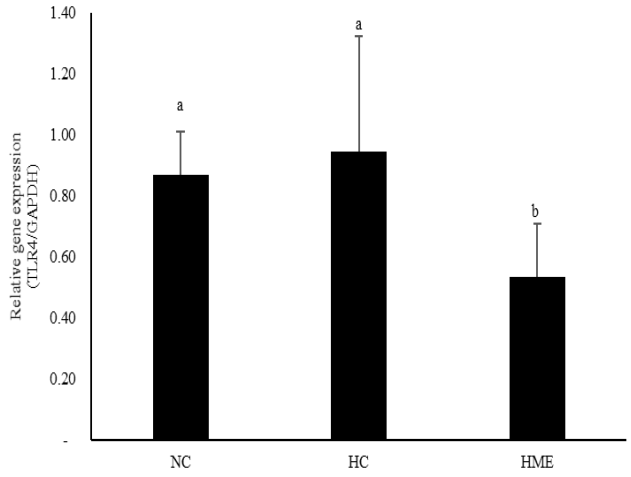

Fig. 6. Effect of moderate intensity exercise on TLR4 in high fat diet induced obese mice. Data represent means±SE. Different alphabet means significant difference.

The study result of IL-10 gene expression was shown in Figure 7. The IL-10 gene expression of high-fat diet control was significantly reduced with respect to the normal diet control group (p=0.00). Gene expression of the anti-inflammatory cytokine IL-10 was persistently elevated in HFD mice as well [28]. However, the high-fat diet with moderate intensity exercise was not significantly increased the IL-10 gene expression with respect to the high-fat diet control group.

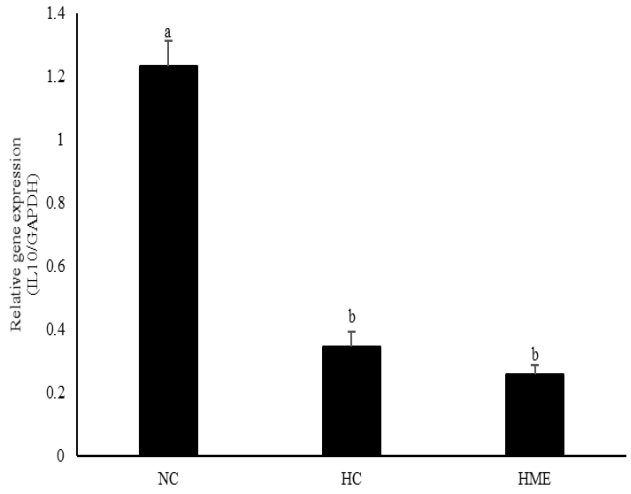

Fig. 7. Effect of moderate intensity exercise on IL-10 in high fat diet induced obese mice. Data represent means±SE. Different alphabet means significant difference.

IL-10 is anti-inflammatory cytokine inflammation normalize the physiological or pathological conditions by inhibiting the function of T cell, monocyte, and macrophage-mediated in several organs and tissues [37]. IL-10 has been distinguished in some liver cells, including hepatocytes, stellate cells, and Kupffer cells, but only partial research that has been accomplished to explore the role of endogenous IL-10 in the progress of NAFLD. Other study suggested that endogenous IL-10 was defensive for hepatic steatosis, but not for attendant insulin resistance in IL10 deficient mice [38]. Another study detected that the inhibition of IL-10 increased the expression of IL-1β, F4/8, TNF-α, IL-6, and reduced insulin signal transduction and steatosis [39]. Our study found that moderate intensity exercise in high-fat-diet-induced obese mice decreased the mRNA expression of IL-10.

IV. Conclusion

Obesity-induced metabolic inflammation in the AT precedes inflammation in the liver, suggesting that the liver does not play a role in the initial development of metabolic inflammation. Modification lifestyle such as increasing physical activity and decreasing fat consumption was already recommended. Furthermore, we show that moderate intensity exercise can eliminate the inflammation marker TNFα and IL-1β. However, it not enough to decrease the TLR4 and increase the IL-10.

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