

Original article

# Regular moderate aerobic exercise improves high-fat diet-induced nonalcoholic fatty liver disease via monoacylglycerol *O*-acyltransferase 1 pathway suppression

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## Abstract

**Purpose:** Monoacylglycerol *O*-acyltransferase 1 (MGAT1) is reported to play a key role in the development of diet-induced nonalcoholic fatty liver disease (NAFLD). Thus, this study investigated the effect of exercise on suppression of the MGAT1 pathway in NAFLD tissue of high-fat diet (HFD)-induced obese rats.

**Methods:** Male Sprague-Dawley rats were fed an HFD containing 45% fat for 6 weeks. Upon confirmation that NAFLD had been induced in the obese animals, they were divided into HFD-fed groups provided with exercise (HFD + EXE) or without exercise (HFD) and a group given dietary adjustment (DA) only, for a further 6 weeks of intervention treatment. The 6-week regular moderate aerobic exercise consisted of an accommodation phase with increasing exercise. Lipid accumulation in the liver tissue was determined by Oil Red O staining. The MGAT1 and liver lipogenic gene mRNA levels were measured by qPCR, and their protein levels by western blot assay.

**Results:** Oil Red O staining showed that NAFLD was successfully induced by HFD-fed. The gene expression of MGAT1 was significantly lower in HFD + EXE than HFD. However, there was no significant difference between HFD + EXE and DA. The protein expression of MGAT1 was significantly lower in HFD + EXE than both HFD and DA. Messenger RNA and protein expression of other lipogenic genes were not different among groups. These data indicate that exercise suppresses MGAT1 pathway regardless of HFD feeding; in part, this effect could be greater than DA.

**Conclusion:** Our data suggest that exercise can improve NAFLD, which is probably due to suppression of MGAT1 pathway.

**Keywords:** Exercise; High-fat diet; Monoacylglycerol *O*-acyltransferase 1; Nonalcoholic fatty liver disease; Obesity

## 1. Introduction

Fatty liver disease is classified into alcoholic fatty liver disease and nonalcoholic fatty liver disease (NAFLD), where the latter is attributed to causes other than excessive alcohol consumption and is most commonly induced by obesity due to westernized dietary patterns, such as a high-fat diet (HFD). Although NAFLD is thought to have a better prognosis than its alcoholic counterpart, long-term epidemiologic and experimental studies have shown that the nonalcoholic fatty liver can undergo fibrosis, which may lead to cirrhosis or liver cancer (hepatocellular carcinoma).<sup>1–6</sup>

Because of its link to obesity, the recommended treatments for NAFLD are weight loss, dietary control, and exercise. In particular, exercise is known to play a role in improving this condition, independent of weight loss or dietary control.<sup>7–10</sup> The prevalence of NAFLD was also found to be low among individuals who performed a large amount of physical activity.<sup>9</sup> There is no dispute in either the medical or the sports sciences communities about the possibility of improving NAFLD through exercise. However, the physiological or molecular biological mechanisms through which exercise improves NAFLD are currently unknown, largely because the molecular biological mechanism of NAFLD progression had for a long time not been elucidated. Recently, however, the molecular pathway for NAFLD progression was identified, wherein the synthesis of triglycerides in the liver was shown to be greatly increased by the high expression of monoacylglycerol *O*-acyltransferase 1 (MGAT1).<sup>11</sup>

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Peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) is known to be activated the expression of lipogenic genes such as sterol regulatory element-binding protein (SREBP1c) and carbohydrate-responsive element binding protein (chREBP) in association with lipid accumulation as a transcription factor. However, PPAR $\gamma$  expression remains very low in the liver. Lee et al.<sup>11</sup> found that PPAR $\gamma$  is highly expressed in the NAFLD in animal model, and the reported for the first time that PPAR $\gamma$  stimulated the synthesis of triglycerides by the expression of MGAT1. In addition, the study confirmed that MGAT1 inhibition significantly reduced the accumulation of triglycerides in the NAFLD. In conclusion, Lee et al.'s<sup>11</sup> study confirmed the NAFLD specific pathway of lipid metabolism and suggested that MGAT1 could be an important target for the treatment of NAFLD.

If exercise positively changes MGAT1 expression, it can provide a molecular mechanism underlying the effect of exercise on the improvement in NAFLD reported in many previous studies.<sup>8–10,12</sup> It can also provide a strong molecular evidence for prescribing exercise as a treatment for NAFLD.

Therefore, the purpose of this study was to investigate whether the effects of exercise on improvement in NAFLD were due to suppression of the MGAT1 pathway. To demonstrate this hypothesis, we first induced NAFLD with an HFD and confirmed the expression of MGAT1. In addition, 6 weeks of intervention was performed to confirm the effect of regular moderate exercise and dietary adjustment (DA). After the intervention, MGAT1 and its transcription factor PPAR $\gamma$  and expression of SREBP1c and chREBP, which decreased in expression relative to MGAT1 in the NAFLD, were analyzed.

## 2. Materials and methods

### 2.1. Experimental design

Three-week-old male Sprague-Dawley rats ( $n = 35$ ) were purchased from Central Laboratory Animal (Seoul, Republic of Korea). All animals were provided *ad libitum* access to both water and feed. The animal studies were approved by the Institutional Animal Care and Use and Committee of Pusan National University (approval number: PNU-2010-000106). The rats were kept in a room maintained at 22°C–24°C with 50%–60% relative humidity and a 12-h light/12-h dark cycle. Twenty-eight of the animals were fed an HFD containing 45% fat (Research Diets, New Brunswick, NJ, USA) for 6 weeks, whereas the remaining 7 rats were fed a normal diet (ND). After this period, 7 of the HFD-fed obese animals and all of the rats in the ND group were humanitarian sacrifice to determine whether NAFLD had been induced, and the related genes were analyzed. After confirming the induction of NAFLD, the remaining 21 HFD-fed rats were divided randomly into 3 groups: the HFD group, the HFD with exercise (HFD + EXE) group, and the dietary adjustment (DA) group (Fig. 1). At this point, the HFD and HFD + EXE groups maintained the same HFD, but the HFD + EXE group was subjected to exercise, whereas the DA group was switched from the HFD back to an ND without exercise. The purpose of this step was to compare the independent effects of exercise and DA in obese rats with induced NAFLD.

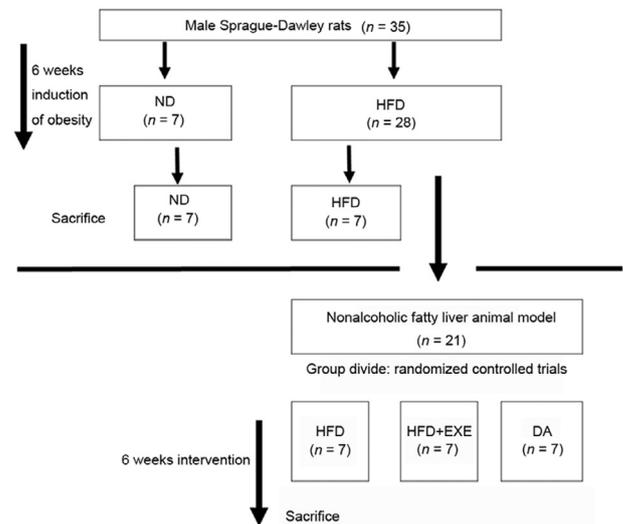


Fig. 1. Experimental design of this study. DA = dietary adjustment; EXE = exercise; HFD = high-fat diet; ND = normal diet.

Therefore, an ND with exercise group, which is a factor well-known to be effective in improving NAFLD, including both ND and exercise, was not included in the intervention group because it was not suitable for this study.

### 2.2. Regular moderate aerobic exercise protocol and body weight and food intake

The HFD + EXE group was subjected to exercise on an animal treadmill (DJ-344; Daejong Instrument Industry, Daejong, Republic of Korea). The 6-week-long exercise regimen consisted of an accommodation phase with increasing exercise intensity (Weeks 1–3, 15 m/min for 30, 45, and 60 min, respectively; and Weeks 4–5 for 20 m/min for 30 or 45 min, respectively), followed by a final 1-week consistent training period (20 m/min for 60 min). Training was conducted 5 times a week between the hour of 6:00 p.m. and 7:00 p.m. As a control, the animals in the other groups were placed on an idle treadmill for the same time period. Before each training session, all exercising rats were allowed a 5-min warm-up phase with a slow increase of speed.<sup>13</sup> The body weight and food intake of each animal were measured weekly throughout the study.

### 2.3. Measurements

#### 2.3.1. Sample collection

Sample tissue collection from the experimental animals was performed at 48 h after the last exercise session to exclude any immediate physiological effects owing to exercise. Feeding was stopped for 10 h before humanitarian sacrifice to exclude physiological effects from the diet. After anesthetizing the rat with ethyl ether, the skin was peeled from the lower abdomen to the cervical vertebra and the liver was excised. The liver was removed, washed in 0.9% saline, and immediately placed in liquid nitrogen. All samples were stored at  $-70^{\circ}\text{C}$  until further analysis.

### 2.3.2. Oil Red O (ORO) staining

ORO staining of the liver tissue was performed to measure the hepatic lipid content. For this experiment, a part of the liver tissue was placed in a mold containing the optimal cutting temperature compound, fixed in formalin, and stored in a deep freezer at  $-70^{\circ}\text{C}$ . Cryo tissue sections were prepared from these frozen liver tissues for fixing onto microscope slides. To prepare the staining solution, 500 mL of 100% propylene glycol (Sigma-Aldrich, St. Louis, MO, USA) was used to dissolve the ORO powder completely. The ORO solution was then filtered through a 25- $\mu\text{m}$  filter paper. Distilled water was added to dilute the 100% propylene glycol to an 85% concentration. The prepared cryo tissue section slides were dried at room temperature for 60 min, and 10% ice-cold formaldehyde was then added for 10 min to fix the tissue onto the slides. Thereafter, each slide was immediately washed with distilled water, dried again at room temperature, and then immersed in 100% propylene glycol for 2–5 min to prevent the presence of water in the ORO staining. After staining for 1 h in a pre-warmed ORO solution at  $60^{\circ}\text{C}$  in a drying oven and then for 5 min in the 85% propylene glycol solution, the slide was washed with distilled water. Hematoxylin solution (Santa Cruz Biotechnology, Heidelberg, Germany) was added for 30 s to stain the nuclei and was then washed away under running tap water for 1 min. The slide was immersed in distilled water to prevent any staining reagent from remaining. Finally, a coverglass was fixed to the slide with an aqueous mounting medium (Dako, Carpinteria, CA, USA) and the tissue was observed under a microscope.<sup>14</sup>

### 2.3.3. Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qPCR) was performed to measure gene expression at the messenger RNA (mRNA) level. The liver tissue was crushed finely on dry ice using a scalpel and then transferred to a 1.5 mL tube. RNA was then isolated using the TRIzol reagent (Life Technologies, Carlsbad, CA, USA). Complementary DNA was synthesized from the RNA, using the PrimeScript II 1st Strand complementary DNA Synthesis Kit (Takara, Tokyo, Japan) according to the manufacturer's instructions. The complementary DNA was mixed respectively with forward and reverse primers of MGAT1, PPAR $\gamma$ , chREBP, and SREBP1c in a 96-well plate containing SYBR<sup>®</sup> Premix Ex Taq (Takara). Each sample was dispensed in triplicate. To normalize the mRNA levels among the different samples, tyrosine 3-mono-oxygenase/tryptophan 5-monooxygenase activation protein, zeta (YWHAZ), known as the most suitable housekeeping gene to NAFLD tissue analyze, was used in the qPCR.<sup>15</sup> Analysis was performed using the ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The primer sequences used in the experiments are shown in Table S1. The cycle threshold values measured after the experiment were analyzed by the relative quantification  $2^{-\Delta\Delta\text{C(T)}}$  method.<sup>16</sup>

### 2.3.4. Western blot

Western blotting was performed to detect the protein levels of PPAR $\gamma$ , MGAT1, and other fatty acid synthesis-related molecules (SREBP1c and chREBP) in the hepatocytes. Liver tissue

was homogenized in protein extraction solution (PRO-PREP; Intron Biotechnology, Seongnam, Republic of Korea). The homogenate was centrifuged at 13,000 rpm for 5 min at  $4^{\circ}\text{C}$ , and the supernatant was collected. The protein concentration was determined using the Bradford protein assay. Twenty micrograms of soluble protein were boiled in Laemmli buffer for 5 min at  $100^{\circ}\text{C}$ , loaded onto a 12% polyacrylamide gel, and electrophoresed for 1.5 h at  $20^{\circ}\text{C}$ . The gel was blotted onto a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) and stained with Ponceau S (Sigma-Aldrich) to confirm the equal loading and transfer of proteins from each lane to the membrane. Blocking of the membrane was carried out for 1 h in 5% skim milk in Tris-buffered saline with 0.05% Tween 20 at room temperature, and it was then probed overnight with primary antibodies at  $4^{\circ}\text{C}$  (Table S2). This step was followed by incubation with the secondary antibodies (Table S2), and the signals were developed using a chemiluminescence reagent. The results were quantitated using the FluorChem HD2 imaging system (Alpha Innotech, San Leandro, CA, USA) and the integrated density value of the bands (3 bands/group) calculated through the program was analyzed 3 times to calculated mean and standard deviation.

### 2.4. Statistical analysis

Statistical analysis was performed using SPSS (Version 23.0; IBM Corp., Armonk, NY, USA) software. An independent samples *t* test was used to compare all differences between the 2 groups (ND, HFD) in the induction of NAFLD. One-way analysis of variance was conducted for comparison among groups (HFD, HFD+EXE, and DA) of intervention stage. Two-way repeated measures analysis of variance and Duncan's *post hoc* test were used to compare body weight changes between groups as time goes by. The results were considered statistically significant at  $p < 0.05$ .

## 3. Results

### 3.1. Body weight gain, food intake, and food efficiency ratio

In the NAFLD induction phase, there were significant differences in the body weight of each group from the third week, becoming more obvious until the 6th week (Fig. S1A). In the first week after start of the exercise and dietary interventions, the effects of exercise (Fig. S1B<sup>a</sup>) began to appear, whereas that of diet did not (Fig. S1B<sup>b</sup>).

The HFD+EXE group had significantly less weight gain than the HFD group ( $p < 0.001$ ). The effect started to appear from the first weeks and continued until the end of the experiment (Fig. S1B<sup>a</sup>). In addition, the DA group showed significantly less weight gain than the HFD group (Fig. S1B<sup>b</sup>). The HFD+EXE group has significantly higher body weight gain than the DA group (Fig. S1B<sup>c</sup>). The difference in mediating significance between the 2 groups did not appear in the second and third weeks, but the difference in weight began again to sharply increase again from the 4th week and the gap widened in the final weight ( $p < 0.001$ ).

Although the food intake by the ND group was higher in the NAFLD induction phase, the high energy density of the HFD

Table 1

Information on weight change, food intake, and food efficiency in the different groups (mean±SD).

	Induced NAFLD with HFD		Intervention			F	Post hoc*
	ND (n = 7)	HFD (n = 28)	HFD (n = 7)	HFD + EXE (n = 7)	DA (n = 7)		
Initial weight (g)	119.90 ± 2.40	119.10 ± 5.94	486.74 ± 14.13	486.45 ± 10.40	486.32 ± 14.82	0	NS
Final weight (g)	417.30 ± 16.09	476.20 ± 17.65 <sup>#</sup>	650.79 ± 24.41	537.7 ± 24.75	608.14 ± 21.62	40.83 <sup>†</sup>	B < C < A
Total weight gain (g)	297.40 ± 16.67	357.00 ± 16.20 <sup>#</sup>	164.04 ± 25.09	51.25 ± 19.61	121.86 ± 13.16	4.56 <sup>‡</sup>	B < C < A
Average weight gain (g/week)	49.50 ± 3.52	59.50 ± 9.15 <sup>#</sup>	27.34 ± 2.34	8.54 ± 2.10	20.30 ± 2.19	57.4 <sup>†</sup>	B < C < A
Food intake (g/week)	167.00 ± 6.57 <sup>#</sup>	142.40 ± 4.36	117.35 ± 9.95	92.59 ± 5.25	175.53 ± 6.63	219.29 <sup>†</sup>	B < A < C
FER <sup>§</sup>	0.30 ± 0.01	0.41 ± 0.00 <sup>#</sup>	0.23 ± 0.03	0.09 ± 0.03	0.10 ± 0.01	48.71 <sup>†</sup>	B < C < A

\* Group name, A = HFD; B = HFD + EXE; C = DA.

<sup>#</sup>  $p < 0.001$ , significantly different between ND and HFD in induced NAFLD with high-fat diet.<sup>†</sup>  $p < 0.001$ , significantly different among HFD, HFD + EXE, and DA in intervention group.<sup>‡</sup>  $p < 0.01$ .<sup>§</sup> Calculated as body weight gain food intake.Abbreviations: DA = dietary adjustment; EXE = exercise; FER = food efficiency ratio, calculated as body weight gain (g/week)/food intake (g/week); HFD = high-fat diet; NAFLD = nonalcoholic fatty liver disease; ND = normal diet; NS = no statistically significant difference at  $p < 0.05$ .

resulted in more significant differences in weight gain and diet efficiency in the rats (Table 1).

### 3.2. Changes in liver lipid accumulation after induction of NAFLD and intervention

Compared with that seen for the ND group, the lipid tissue of the HFD group was stained with a widely spread red color, indicating that obesity and NAFLD had been induced by the HFD (Fig. 2A). After intervention in the NAFLD-induced obese rats, a large amount of lipid accumulation was observed in the HFD group compared with that in the other 2 groups, indicating that the NAFLD status was maintained during the 12 weeks of the HFD intake. Conversely, in the HFD + EXE and DA groups, respectively, relatively fewer red stained parts were observed and the NAFLD was improved relative to that in the HFD group. Comparisons between the HFD + EXE and DA groups led to the conclusion that DA could be more effective than exercise for improving the fatty liver. However,

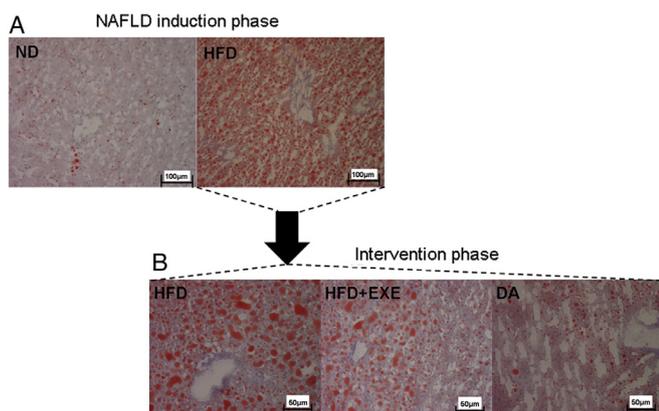


Fig. 2. (A) Oil red O (ORO) staining (original magnification  $\times 100$ ) of liver tissue from normal diet (ND,  $n = 7$ ) and high-fat diet (HFD,  $n = 28$ ) induction and intervention measures. (B) ORO staining (original magnification  $\times 50$ ) of rat liver tissue after exercise (HFD + EXE,  $n = 7$ ) and diet adjustment (DA,  $n = 7$ ) interventions. The control group after the measures was set as the HFD ( $n = 7$ ) group.

because exercise was performed in conjunction with continued HFD feeding in the HFD + EXE group, it was confirmed that the NAFLD was also improved by exercise alone (Fig. 2B).

### 3.3. qPCR analysis of changes in MGAT1 and lipogenic gene mRNA expression after NAFLD induction and intervention

The lipogenic genes were compared after the obesity and NAFLD induction stages. There was a significant difference in MGAT1 and chREBP expression between the 2 groups, where the expression of MGAT1 was significantly higher in the HFD group, suggesting that the diet had induced abnormal liver metabolism. However, despite the increase of MGAT1 by PPAR $\gamma$ , there was no significant difference in PPAR $\gamma$  levels between the 2 groups (Fig. 3). In addition, chREBP was higher

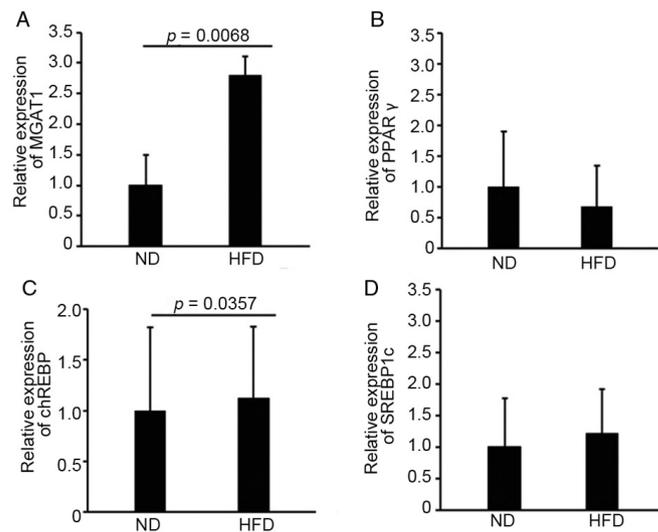


Fig. 3. Changes in genes expressions involved in hepatic lipogenesis after 6 weeks of HFD or ND feeding. The expression of MGAT1 was significantly increased in the HFD group, indicating abnormal fatty acid metabolism. chREBP = carbohydrate-responsive element binding protein; HFD = high-fat diet; MGAT1 = monoacylglycerol *O*-acyltransferase 1; ND = normal diet; PPAR $\gamma$  = peroxisome proliferator-activated receptor gamma; SREBP1c = sterol regulatory element-binding protein 1.

in the HFD group, suggesting that the classical pathway for the metabolism of some carbohydrates has a role in abnormal lipogenesis. There were significant differences in MGAT1 and PPAR $\gamma$  expression among the 3 groups after intervention (Fig. 4). MGAT1 was significantly lower in the HFD+EXE group than in the HFD group, suggesting that exercise inhibited MGAT1 independently of diet (Fig. 4A). For PPAR $\gamma$ , however, it is presumed that the transcription factor binds to other specific lipogenic genes as well, because PPAR $\gamma$  expression was not significantly different between the HFD+EXE and DA groups (Fig. 4B). However, the expression of chREBP and SREBP1c, the genes involved in carbohydrate-dependent lipid synthesis in the liver, was not significantly different between the groups (Fig. 4C, D).

### 3.4. Changes in MGAT1 and lipogenic protein expression after NAFLD induction and intervention

Western blot analysis showed that the expression of lipogenic protein in the NAFLD induction phase of the protein was similar to the qPCR results, and higher expression of MGAT1 and PPAR $\gamma$  was observed in the HFD group (Fig. 5B, C). We also observed differences in the amount of protein expressed in the lipogenic protein after exercise and dietary intervention (Fig. 6A). In the HFD group, the expression of MGAT1 was higher than HFD+EXE group. The DA group showed less expression of MGAT1 than the HFD group, but there was no significant difference. Also, encouragingly, the expression of MGAT1 was significantly lower in the HFD+EXE group than in the DA group ( $p < 0.05$ ; Fig. 6B). This finding suggests that DA alone does not significantly inhibit MGAT1 protein expression without exercise. It may also be because there was no dramatic difference in caloric restriction owing to the *ad libitum* dietary intake in this experiment. In the case of PPAR $\gamma$ , protein

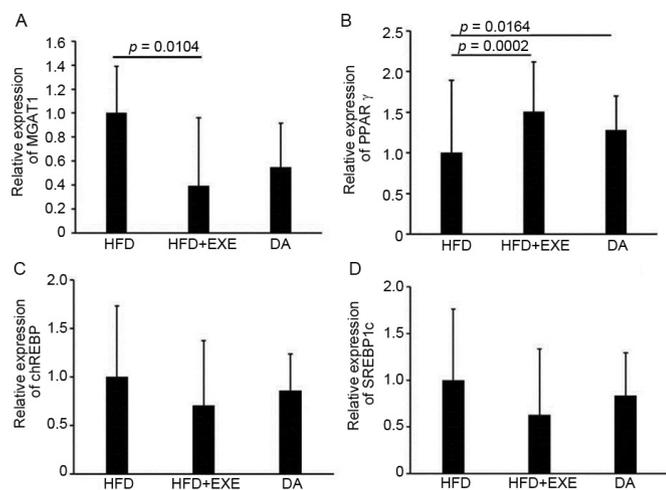


Fig. 4. Changes in genes expressions involved in hepatic lipogenesis after exercise HFD+EXE or DA in rats fed an HFD. Exercise and DA reduced the MGAT1 expression, with NAFLD improvement. chREBP = carbohydrate-responsive element binding protein; DA = dietary adjustment; HFD = high-fat diet; HFD + EXE = high-fat diet with exercise; MGAT1 = monoacylglycerol *O*-acyltransferase 1; PPAR $\gamma$  = peroxisome proliferator-activated receptor gamma; SREBP1c = sterol regulatory element-binding protein 1.

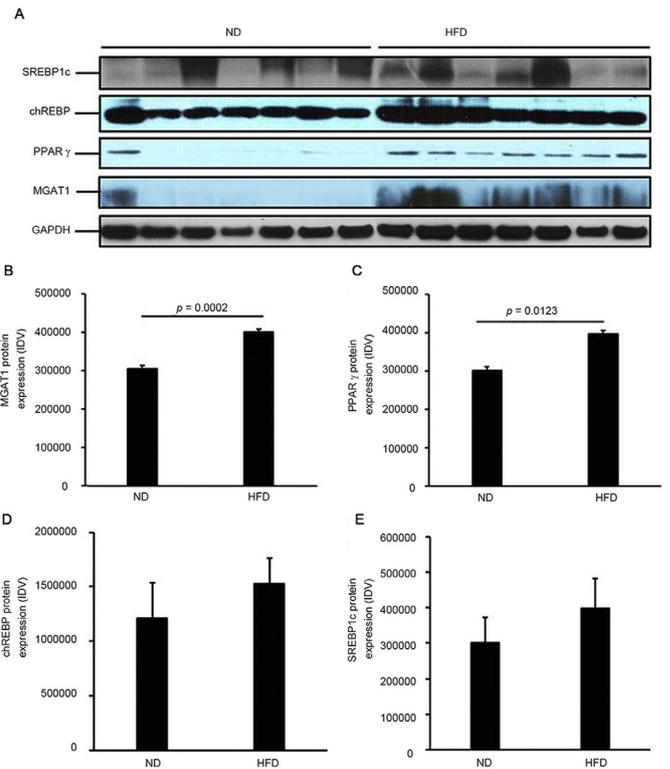


Fig. 5. Expression of lipogenic proteins after NAFLD induction by 6 weeks of HFD feeding. (A) Representative images of western blot protein bands. (B–E) The integrated density values of the western blot bands were measured with FluorChem HD2 and plotted (ND,  $n = 7$ ; HFD  $n = 7$ ) as described in materials and methods section. chREBP = carbohydrate-responsive element binding protein; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; HFD = high-fat diet; IDV = integrated density value; MGAT1 = monoacylglycerol *O*-acyltransferase 1; NAFLD = non-alcoholic fatty liver disease; ND = normal diet; PPAR $\gamma$  = peroxisome proliferator-activated receptor gamma; SREBP1c = sterol regulatory element-binding protein 1.

expression was higher in the HFD+EXE and DA groups than in the HFD group. The HFD+EXE group was also significantly higher than the DA group ( $p < 0.05$ ). The high PPAR $\gamma$  expression in the HFD+EXE group seems to be very significant and seems to be independently increased by exercise (Fig. 6C). There was no difference in the expression level of chREBP and SREBP1c (Fig. 6D, E).

## 4. Discussion

The fact that the expression of MGAT1 is important in NAFLD, as identified in previous studies,<sup>11</sup> was again confirmed in this study. However, a new finding in the present study is that MGAT1 can be suppressed through chronic exercise independent, because given that the exercise-induced MGAT1-lowering effect occurred while the animals were maintaining an HFD. The least weight gain was observed in the HFD+EXE group, in which food intake and diet efficiency were also the least, suggesting that weight loss may be associated with energy expenditure when exercise is performed and, hence, dietary intake may also decrease. Previous studies have shown that exercise can

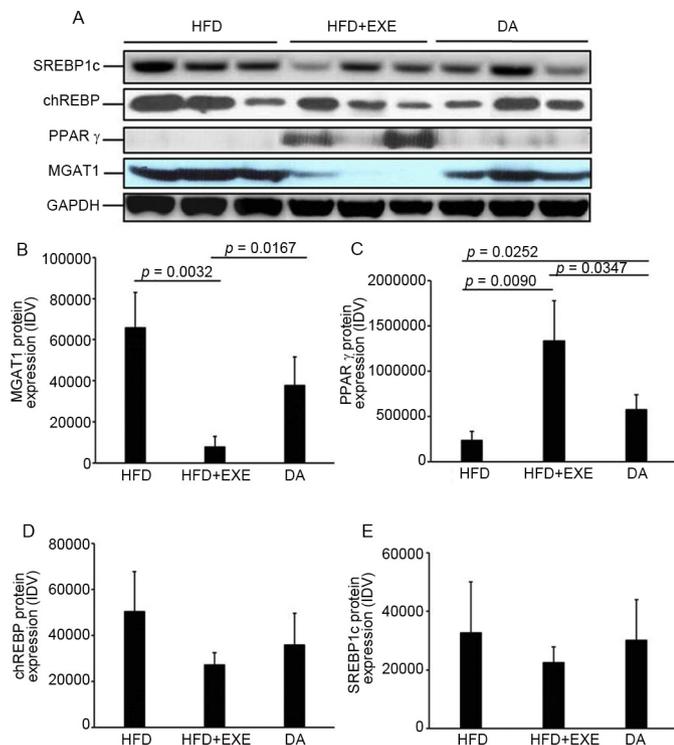


Fig. 6. Expression of lipogenic protein after exercise (HFD+EXE) or diet adjustment (DA) interventions. (A) Western blot analysis. (B–E) The integrated density values of the western blot bands were measured with FluorChem HD2 and plotted (for each group,  $n = 3$ , repeated 3 times). chREBP = carbohydrate-responsive element binding protein; DA = dietary adjustment; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; HFD = high-fat diet; HFD+EXE = high-fat diet with exercise; IDV = integrated density value; MGAT1 = monoacylglycerol *O*-acyltransferase 1; PPAR $\gamma$  = peroxisome proliferator-activated receptor gamma; SREBP1c = sterol regulatory element binding-protein 1.

decrease dietary intake as well as increase energy consumption, and this finding was also true in this study.<sup>17,18</sup> Exercise and DA are effective for weight loss, but the presence or absence of exercise is important for weight loss when *ad libitum* are used regardless of the energy intake components. One study showed that when *ad libitum* diets were administered (i.e., a high-carbohydrate diet similar to the *ad libitum* diet of most HFDs), there was a significant decrease in body weight and a significant increase in insulin sensitivity in a single-exercise group.<sup>19</sup>

We confirmed that lipid accumulation in the liver tissue was actually decreased, together with the weight loss. Although exercise and DA both decreased lipid accumulation in the liver, the latter was found to be more effective in this regard. These results show that, in the HFD + EXE group, most of the energy sources used in the body of the exercising animals were fatty acids. The fact that this group still showed a decrease in hepatic lipid accumulation despite consuming the HFD means that exercise independently decreased lipid accumulation in the liver and improved NAFLD. Lipotoxicity means that the excess accumulation of lipid in the non-adipose tissue (liver, muscle, heart, pancreas, and blood vessels) cells exceeds the capacity of the lipid storage to cause cell dysfunction.<sup>20</sup> However, as with the results of this study, our previous

study in the same animal as this study also showed that lipid accumulation in muscle decreased independently of a HFD.<sup>21</sup> Thus, this study, along with our previous study, strongly supports that exercise is effective in improving lipotoxicity.

We have confirmed that weight loss and decreased hepatic lipid accumulation are indeed caused by the suppression of MGAT1. We analyzed MGAT1 and its transcription factor, PPAR $\gamma$ , together with chREBP and SREBP1c, which have been used primarily to explain lipid accumulation in the liver before the MGAT1 pathway was identified.<sup>22</sup> At the mRNA level, it was confirmed that the HFD induction of obesity elicited a significant increase of MGAT1, resulting in a fatty liver. However, although MGAT1 was regulated by PPAR $\gamma$ , both the mRNA and protein expression levels of PPAR $\gamma$  were higher in the HFD + EXE and DA groups.

Previous studies have shown that PPAR $\gamma$  is greatly increased by exercise, and it seems not a special phenomenon.<sup>23</sup> It is known that PPAR $\gamma$  increases transiently owing to exercise-induced oxidative stress in the muscle cells, and its expression increases with increasing oxidative capacity even after long-term exercise.<sup>24,25</sup> In addition, it has been shown that PPAR $\gamma$  may be increased through exercise in many other cells, such as liver cells.<sup>26</sup> The risk of NAFLD can be increased by fatty acid  $\beta$ -oxidative damage or *de novo* lipogenesis.<sup>27</sup> Increased PPAR $\gamma$  in the exercise group seems to increase  $\beta$ -oxidation of fatty acids, especially because exercise increases this process through the expression of PPAR $\gamma$ . The fact that PPAR $\gamma$  is expressed mainly in adipose tissue is supported by the fact that the expression was also increased in the liver tissue of experimental animals.<sup>28</sup>

However, the fact that MGAT1 was significantly reduced in the HFD + EXE group, as opposed to the increase in PPAR $\gamma$ , clearly indicates that exercise is involved when PPAR $\gamma$  modulates MGAT1. However, to understand the molecular pathways that change in the liver when exercise is performed, it is necessary to further analyze fatty acid synthesis, triglyceride metabolism, and the genes associated with transport proteins through microarray and other experimental methods. As discussed elsewhere in this article, not only did the HFD + EXE group not deteriorate, even though the HFD was continued, but the rats improved through exercise. Therefore, aerobic exercise can inhibit MGAT1 independently and alleviate the NAFLD condition without dietary restriction. This finding means that regular exercise is as important as dietary control in the treatment of NAFLD.

## 5. Conclusion

Our study confirmed that the specific effect of regular moderate aerobic exercise is to greatly inhibit the NAFLD tissue expression of MGAT1. In addition, it was confirmed MGAT1 was inhibited by regular moderate exercise in spite of eating HFD.

## Authors' contributions

KWB carried out gene expression and protein expression studies, and drafted manuscript; JAG carried out the

statistical analysis; JJP conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

### Competing interests

The authors declare that they have no competing interests.

### Supplementary materials

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.jshs.2018.09.001>.

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