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

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ABSTRACT

Purpose: *Recent studies suggest that exchange of macrophage phenotype (M1/M2) in adipose tissue is associated with chronic low-grade inflammation in obesity. M1 macrophages enhance a chronic inflammatory state in adipose tissues, whereas M2 macrophages inhibit it. Although exercise training might inhibit pro-inflammatory cytokine gene expression in adipose tissue, it remains unclear whether exercise training affects the phenotypic switch of macrophage polarization in adipose tissue. Therefore, we investigated the effect of exercise training on the macrophage phenotypic switch in adipose tissue in high-fat-induced obese mice.* ***Methods:*** *Male C57BL/6 mice were divided into four groups; normal diet (ND) control (n=7), ND exercise (n=7), high-fat-diet (HFD) control (n=12), and HFD exercise (n=12) groups. All exercised mice ran on a treadmill at 12-20 m/min for 60 min/day for 16 weeks. Tumor necrosis factor (TNF)-α, interleukin (IL)-6, F4/80, monocyte chemotactic protein (MCP)-1, CXCL14, inter-cellular adhesion molecule (ICAM)-1, vascular-cellular adhesion molecule (VCAM)-1, CD11c, CD163 and toll-like receptor (TLR)4 mRNA expressions in adipose tissue were evaluated by real time-RT-PCR.* ***Results:*** *In HFD mice, exercise training did not induce loss of body or adipose tissue mass, exercise training nevertheless markedly inhibited TNF-α and F4/80 mRNA expression in adipose tissue. The exercise training attenuated HFD-induced increase in ICAM-1 mRNA expression, but not MCP-1, CXCL14 and VCAM-1 mRNA expressions. In addition, increased*

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CD11c mRNA expression, which is a M1 macrophage specific marker, with HFD treatment was attenuated by exercise training. In contrast, although the mRNA expression of CD163, a M2 macrophage specific marker, in adipose tissue was significantly decreased by HFD, the exercise training significantly increased its expression. Also, the higher mRNA expression of TLR4, which induces pro-inflammatory cytokine production after fatty acid recognition, was strongly inhibited by the exercise training in HFD mice. ***Conclusion:*** *Exercise training might induce the phenotypic switching from M1 macrophage to M2 macrophage in obese adipose tissue besides inhibiting M1 macrophage infiltration into adipose tissue. Therefore, chronic exercise might contribute to inhibit inflammation in adipose tissue via down regulation of TLR4.*

Key words: F4/80, CD11c, CD163, TLR4, real-time PCR

INTRODUCTION

It has been reported that obesity and inactivity are associated with chronic inflammatory diseases, such as type II diabetes and arteriosclerosis (1, 2). In fact, high plasma concentrations of pro-inflammatory cytokines (e.g. tumor necrosis factor (TNF)-α and interleukin (IL)-6) in type II diabetes patients are observed (3), and these cytokines contribute insulin resistance (4). Recent studies have been conducted to examine the influence of obesity on inflammation. There is evidence that the expression of pro-inflammatory cytokines, chemokines and cell adhesion molecules in adipose tissue are increased in obese mice (5-8). In addition, macrophages, which infiltrate adipose tissue in obesity, are known to regulate the inflammatory state of adipose tissue (9-13). Furthermore, a recent study has reported that the inflammation state of adipose tissue was associated with a phenotypic switch of macrophage polarization in adipose tissue (14). High-fat-diet-induced obesity in mice enhances a phenotypic switching from M2 macrophage to M1 macrophage in adipose tissue (15). Macrophage activation has been operationally defined into two separate polarization states, M1 and M2 (16). The M1 macrophage produces TNF-α, IL-6 and nitric oxide. In contrast, the M2 macrophage produces anti-inflammatory cytokines and arginase. Therefore, it can be understood that M1 macrophages induce chronic inflammatory states, and M2 macrophages decrease these states in adipose tissue.

Chronic exercise is effective in prevention and improvement of type II diabetes (17), induces down-regulation of plasma pro-inflammatory cytokines and up-regulation of anti-inflammatory cytokines (18). In fact, recent studies have shown that chronic exercise enhanced down-regulation of TNF-α gene expression in adipose tissue of obese mice (19, 20). Since it is well known that pathogen and intra- or extra-cellular molecule recognition by toll-like receptors such as Toll-like receptor 4 (TLR4), induces NF-κB activation and then TNF-α mRNA expression (21), one possibility is that exercise training-induced TNF-α suppression in obese mice might be regulated by down-regulation of TLR4. Song et al. (22) observed that the mRNA level of TLR4 was enhanced in adipose tissues of obese mice, and activation of TLR4 with free fatty acids stimulated NF-κB signaling and expression of TNF-α and IL-6 in adipocytes (22). Also, Shi et al. (23)

reported that TLR4 recognizes free fatty acids, and then induces pro-inflammatory cytokine and adhesion molecule production. In a review by Gleeson et al. (24), it was noted that cell-surface TLR4 expression and monocyte pro-inflammatory cytokine production capacity in physically active subjects are lower than in physically inactive subjects although the precise physiological stimulus mediating an exercise-induced decrease in cell-surface TLR expression (and TLR mRNA expression) is not known. Accordingly, it is possible that chronic exercise training-attenuated inflammation in adipose tissue occurs by both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. However, it remains unclear if this is the mechanism of down-regulation of pro-inflammatory cytokine gene expression that is brought about by chronic exercise training.

Therefore, we investigated the effects of chronic exercise training on M1 macrophage infiltration into adipose tissue and the phenotypic switch of macrophage polarization in high-fat-diet-induced obese mice. We hypothesized that chronic exercise training would result in decreased macrophage infiltration and a shift of the macrophage phenotype from M1 to M2 in adipose tissue.

MATERIALS & METHODS

Animals and diets

Male C57BL/6 mice (n =38) were purchased from Clea Japan (Osaka, Japan) at 4 weeks of age, and the four animals were housed together in one cage (27 x 17 x 13 cm) in controlled environment under a light-dark cycle (lights on at 20:00 and off at 08:00). The experimental procedures followed the Guiding Principles for the Care and Use of Animals in the KUMW and were approved by the Kawasaki University Medical Welfare Institutional Animal Care and Use Committee (#08-020). All mice were randomly divided into four groups: normal diet (ND) control (n=7), ND exercise (n=7), high-fat-diet (HFD) control (n=12), or HFD exercise (n=12) group. All HFD mice were rendered obese by the HFD (D12492, Research Diets, New Brunswick, NJ) contained with 60 % from fat, 20% from protein and 20% from carbohydrate (of total calories) starting at 4 weeks of age for 16 weeks. All ND mice were fed a standard normal diet contained with 13% from fat, 26% from protein and 60% from carbohydrate (MF, Oriental Yeast, Tokyo, Japan). All groups were allowed to eat food freely. Food ingestion and body weight were measured weekly.

Exercise training protocol

All exercise mice were trained on a motorized treadmill (Natsume, Kyoto, Japan) for 60 min/day at running speeds of 12-20 m/min, 5 times/week, for 16 weeks. Electric shock was not used during the treadmill run. All control mice were housed in cages. Three days after the final exercise training, the exercise trained and untrained mice were sacrificed under light anesthesia with the inhalant Isoflurane, and then several tissues were collected.

Real-time quantitative PCR

To measure mRNA expressions of TNF-α, IL-6, F4/80, monocyte chemotactic protein (MCP)-1, CXCL14, inter-cellular adhesion molecule (ICAM)-1, vascular-

cellular adhesion molecule (VCAM)-1, CD11c, CD163 and TLR4 in adipose tissue, epididymal fat pads in mice were quickly immersed in RNAlater (Applied Biosystems, Carlsbad, CA) and stored at –80°C. Total RNA was extracted using Trizol reagent (Invetrogen, Carlsbad, CA) and RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions and assessed for purity using the NanoDrop system (NanoDrop Technologies, Wilmington, DE). Total mRNA was reverse transcribed to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. RT-PCR was performed using the Fast 7500 real-time PCR system (Applied Biosystems) using Power SYBR® Green PCR Master Mix kits (Applied Biosystems). The thermal profiles consisted of 10 min at 95°C for denaturing followed by 40 cycles of 95°C for 15 s, annealing at 60°C for 1 min.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as the housekeeping gene, and all data are represented relative to its expression (i.e., using standard curve methods) as fold change from ND control group. Specific PCR primer pairs for each studied gene are shown in Table 1.

Table 1. Primer sequences for real-time RT-PCR analysis

gene	forward	reverse
GAPDH	TGAAGCAGGCATCTGAGGG	CGAAGGTGGAAGAGTGGGAG
TNF-α	CCTCCCTCTCATCAGTTCTA	ACTTGGTGGTTTGCTACGAC
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
MCP-1	CTTCTGGGCCTGCTGTTCA	CCAGCCTACTCATTGGGATCA
CXCL14	CCAAGATTCGCTATAGCGAC	CCTGCGCTTCTCGTTCCAGG
ICAM-1	CCTGATGGGCAGTCAACAGCTA	ACAGCTGGCTCCCGTTTCA
VCAM-1	CTTCATCCCCACCATTGAAG	TGAGCAGGTCAGGTTCACAG
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
CD163	GGGTCATTCAGAGGCACACTG	CTGGCTGTCCTGTCAAGGCT
TLR4	ATGGCATGGCTTACACCACC	GAGGCCAATTTTGTCTCCACA

Statistical analysis

Data are presented as means ± SEM. Gene expressions were analyzed using a two-way analysis of variance (ANOVA) with post-hoc Tukey HSD. All analyses were performed using SPSS V17.0 (SPSS, Chicago, IL). Statistical significance was set at $p<0.05$.

RESULTS

Food intake, which was measured in terms of energy intake, varied as a main effect of diet: $F(1,66)=89.43$, $p<0.01$, but the ANOVA revealed no statistically significant effect for exercise training: $F(1,66)=1.39$, not significant (n.s.) and diet x exercise interaction: $F(1,66)=0.218$, n.s. Thus, in this study, the exercise training did not affect the food intake in both ND and HFD mice (Table 2). In addition,

body mass was also affected by diet: $F(1,34)=54.18$, $p<0.01$, but the ANOVA revealed no statistically significant effect for exercise training: $F(1,34)=1.98$, n.s. and diet x exercise interaction: $F(1,34)=0.24$, n.s. The change in epididymal fat mass varied as an effect of diet: $F(1,34)=111.92$, $p<0.01$, but the exercise training: $F(1,34)=0.32$, n.s. and interaction: $F(1,34)=0.157$, n.s. were not statistically significant.

Table 2. The comparsion of food intake, body mass and fat mass between normal (ND) and high fat (HFD) diets in control and exercise trained mice

	ND		HFD	
	control	**exercise**	**control**	**exercise**
Caloric intake (kcal/week)**	**73.2 ± 1.0**	**70.5 ± 2.2**	**88.6 ± 2.0**	**87.4±1.7**
Body mass (g)**	**27.3 ± 0.6**	**25.8 ± 1.0**	**40.4 ± 1.5**	**37.2 ± 1.5**
Epididymal fat mass (g)**	**0.58 ± 0.02**	**0.43 ± 0.01**	**2.12 ± 0.06**	**2.09±0.08**

The values are the means ± SEM. ** p<0.01: effect of the diets

To identify the effect of exercise training on HFD-induced inflammation, we examined gene expressions of TNF-α and IL-6 as key inflammatory markers in adipose tissue (Fig. 1). TNF-α mRNA expression was significantly affected by diet and the exercise training [an effect of diet: $F(1,32)=14.96$, $p<0.01$, exercise training: $F(1,32)=4.71$, $p<0.05$, and diet x exercise training interaction: $F(1,32)=2.60$, n.s.]. Post-hoc comparisons revealed that, in the control condition, TNF-α mRNA expression in HFD mice was significantly higher than that in ND

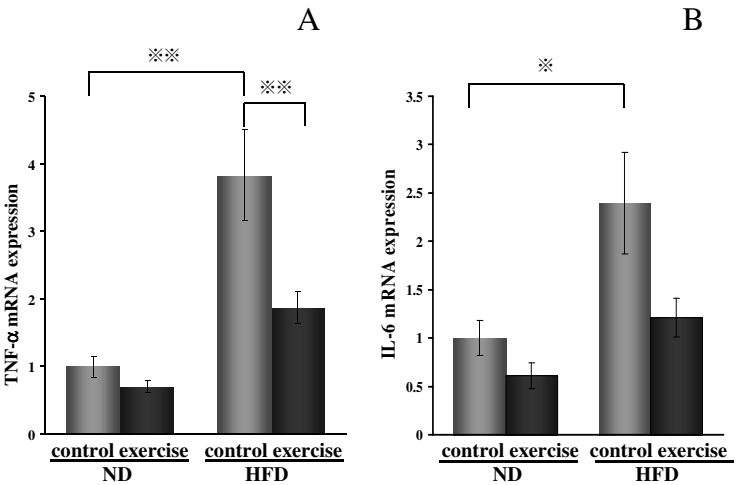

Fig. 1 Effects of exercise training on TNF-α (A) and IL-6 (B) mRNA expression in adipose tissue in both normal- and high-fat-diet fed mice. The values are the means ± SEM. ND: normal diet and HFD: high-fat-diet. *p<0.05 and **p<0.01.

mice (p<0.01). However, the higher expression of TNF-α mRNA was significantly attenuated by exercise training in HFD mice (p<0.01, Fig. 1A). In addition, IL-6 mRNA expression was also affected by the diets [an effect of diet: $F(1,32)=5.88$, p<0.05, exercise training: $F(1,32)=3.62$, n.s., and diet x exercise training interaction: $F(1,32)=1.24$, n.s.]. IL-6 mRNA expression in adipose tissue was also increased by HFD treatment in the control condition (p<0.05, Fig. 1B). As an ANOVA result, although the exercise training did not significantly affect IL-6 mRNA expression (p=0.066), its level in the HFD exercise group decreased (~51% compared with the HFD control group).

F4/80 mRNA expression, reflecting the presence of monocytes and macrophages, varied as an effect of diet: $F(1,32)=21.37$, p<0.01, exercise training: $F(1,32)=7.68$, p<0.01, and diet x exercise training interaction: $F(1,32)=4.93$, p<0.05. Expression of F4/80 mRNA was significantly increased in the HFD control group compared with the ND control group (p<0.01), and the high expression of F4/80 mRNA in HFD control mice was significantly decreased by exercise training (p<0.01, Fig. 2). Therefore, exercise training might reduce macrophage infiltration of adipose tissues in HFD mice.

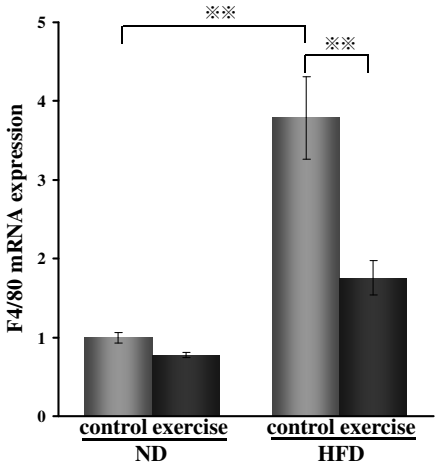

Fig. 2 Effects of exercise training on F4/80 mRNA expression in adipose tissue in both normal- and high-fat-diet-fed mice. The values are the means ± SEM. ND: normal diet and HFD: high-fat-diet. **p<0.01.

The results of mRNA expressions of macrophage chemokines (MCP-1 and CXCL14) are shown in Fig. 3A and B. For both MCP-1 and CXCL14 mRNA expressions, two-way ANOVA revealed no statistically significant effect for exercise training, but there were a statistically significant effect for diet [MCP-1: an effect of the diet: $F(1,32)=28.03$, p<0.01, exercise training: $F(1,32)=2.32$, n.s., and diet x exercise training interaction: $F(1,32)=1.99$, n.s., and CXCL14: an effect of diet: $F(1,32)=5.18$, p<0.05, exercise training: $F(1,32)=3.16$, n.s., and diet x exercise training interaction: $F(1,32)=2.24$, n.s.]. Post-hoc comparisons revealed significant differences between the ND control group and the HFD control group (MCP-1; p<0.01, and CXCL14; p<0.05, respectively).

In addition, Fig. 3 also shows adhesion molecules (ICAM-1; Fig. 3C and VCAM-1; Fig. 3D, respectively) mRNA expression in adipose tissue. For ICAM-1 mRNA, two-way ANOVA indicated an effect of diet: $F(1,32)=25.65$, p<0.01, exercise training: $F(1,32)=26.98$, p<0.01, and diet x exercise training interaction: $F(1,32)=0.00$, n.s. There were statistically significant differences between the ND control and the ND exercise (p<0.05), the ND control and the HFD control (p<0.01), and the HFD control and the HFD exercise groups (p<0.01), respectively. For VCAM-1 mRNA, two-way ANOVA revealed no statistically significant

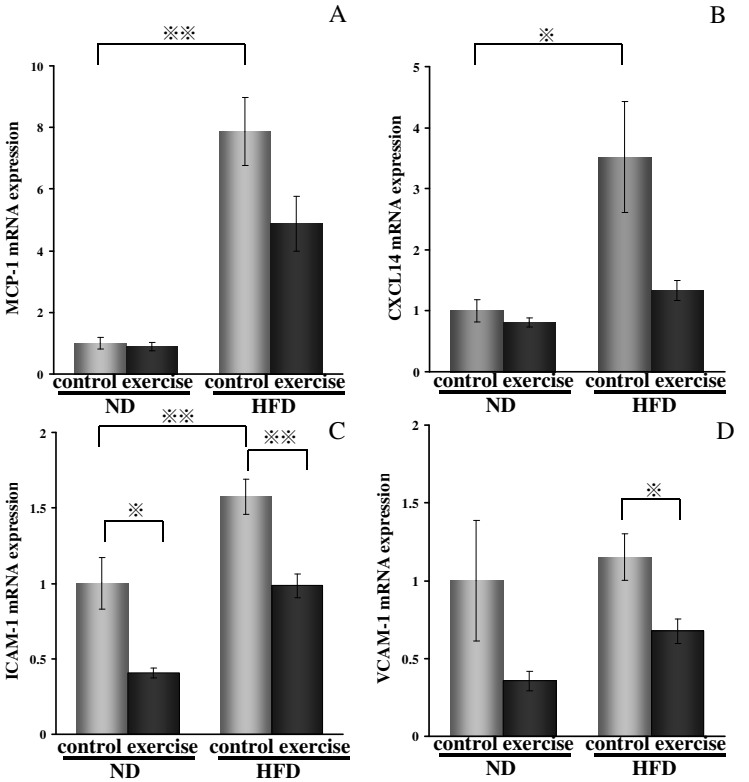

Fig. 3 Effects of exercise training on mRNA expression of the chemokines MCP-1 (A) and CXCL14 (B), and adhesion molecules ICAM-1(C) and VCAM-1 (D) in adipose tissue in both normal diet- and high-fat-diet-fed mice. The values are the means ± SEM. ND: normal diet and HFD: high-fat-diet. *p<0.05 and **p<0.01.

effect of diet: $F(1,32)=1.65$, n.s., but there were a statistically significant effects for exercise training: $F(1,32)=9.25$, $p<0.01$, and diet x exercise training interaction: $F(1,32)=0.21$, n.s. Post-hoc comparisons revealed a significant difference between the HFD control and the HFD exercise groups ($p<0.05$).

To assess the effects of exercise training on HFD-induced phenotypic switch in adipose tissue macrophage polarization, we examined gene expression of M1 and M2 macrophage markers in adipose tissue. As the result of two-way ANOVA, the expression of CD11c mRNA, reflecting the presence of M1 macrophage, varied as an effect of diet: $F(1,32)=16.11$, $p<0.01$, exercise training: $F(1,32)=4.47$, $p<0.05$, and diet x exercise training interaction: $F(1,32)=3.97$, n.s. (Fig. 4A). Post-hoc comparisons revealed that CD11c mRNA expression in the HFD control group was significantly higher than that in the ND control group ($p<0.01$). However, the expression in the HFD exercise group was significantly lower than that in the HFD control group ($p<0.01$). Moreover, there were statistically significant differences in CD163 mRNA expression, reflecting the presence of M2 macrophage, in adipose tissue due to diet and diet x exercise training interaction

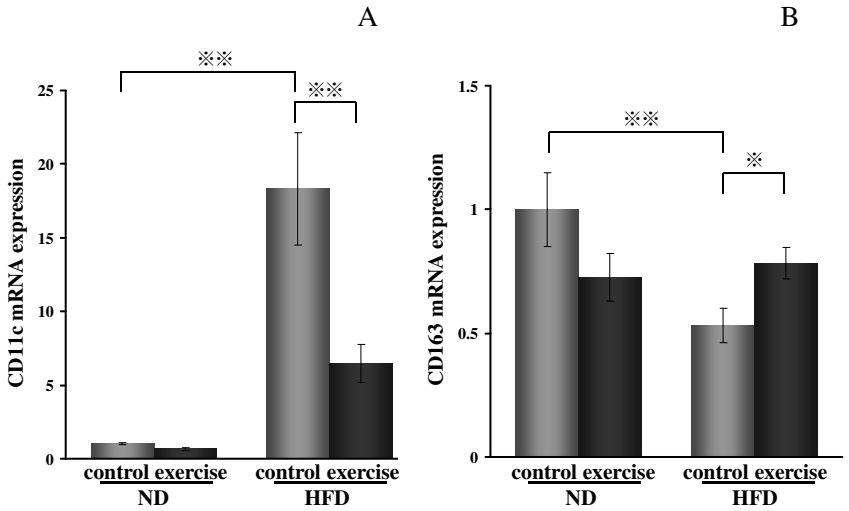

Fig. 4 Effects of exercise training on CD11c as a M1 marker (A) and CD163 as a M2 marker (B) mRNA expression in adipose tissue in both normal- and high-fat-diet-fed mice. The values are the means ± SEM. ND: normal diet and HFD: high-fat-diet. *p<0.05 and **p<0.01.

[an effect of diet: $F(1,31)=7.26$, $p<0.05$, exercise training: $F(1,31)=0.46$, n.s., and diet x exercise training interaction: $F(1,31)=10.74$, $p<0.01$]. Post-hoc comparisons revealed that the expression of CD163 mRNA in HFD control mice was significantly lower than that in ND control mice ($p<0.01$). However, the expression in the HFD exercise group was significantly higher than that in the HFD control group ($p<0.05$, Fig. 4B). These data demonstrate that chronic exercise might induce a decrease in M1 macrophages and an increase in M2 macrophages in adipose tissue in HFD mice.

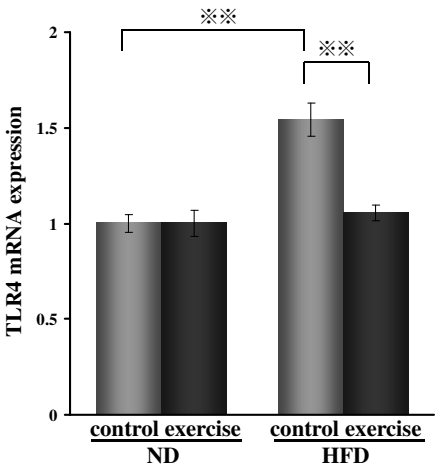

Fig. 5 Effects of exercise training on TLR4 mRNA expression in adipose tissue in both normal diet- and high-fat-diet-fed mice. The values are the means ± SEM. ND: normal diet and HFD: high-fat-diet. **p<0.01.

Interestingly, the mRNA expression of TLR4, which induces production of pro-inflammatory cytokines after recognition of a fatty acid, varied as an effect of diet: $F(1,32)=15.91$, $p<0.01$, exercise training: $F(1,32)=10.42$, $p<0.01$, and diet x exercise training interaction: $F(1,32)=10.52$, $p<0.01$ (Fig.5). Post-hoc comparisons revealed that there

were statistically significant differences between ND control and HFD control ($p<0.01$), and HFD control and HFD exercise groups ($p<0.01$), respectively.

DISCUSSION

Previous studies have shown that voluntary physical activity and forced treadmill exercise training inhibit the expression of pro-inflammatory cytokines in adipose tissue of obese mice (19, 20). These studies concluded that the exercise-induced loss of body mass might be the important factor in the reduction of inflammation, because obese mice exhibited enhanced pro-inflammatory cytokine expression in adipose tissue (5-8). However, in this study, exercise training did not induce the loss of body and epididymal fat mass in HFD mice: the exercise training nevertheless markedly inhibited TNF-α mRNA expression in adipose tissue. Although we can only speculate about the reason for this, our results suggest that exercise training may directly attenuate pro-inflammatory cytokine expression in adipose tissue without requiring exercise-induced loss of overweight. Our results agree with the findings of the other recent studies which have reported that chronic exercise enhances down-regulation of TNF-α gene expression in adipose tissue of obese mice (19, 20).

Furthermore, we observed that higher expression of F4/80 mRNA in adipose tissue was greatly attenuated by exercise training in obese mice. Previous studies have shown that the high expression of pro-inflammatory cytokines after high-fat-diet treatment were attenuated by inhibition of macrophage infiltration in obese mice (9, 10, 25). In fact, increase in F4/80 mRNA expression in adipose tissue relates well to macrophage (Mac3- or CD11b-stained cell) infiltrations into adipose tissue (9). Moreover, Kamei et al. (11) indicated that acceleration of macrophage infiltration into adipose tissue induced high expression of pro-inflammatory cytokines even in normal (non-obese) mice. Therefore, obesity-induced macrophage infiltration into adipose tissue is an important factor for pro-inflammatory cytokine expression, and may be decreased by chronic exercise training. The factors affecting the inhibition of macrophage infiltration into adipose tissue by moderate exercise are presently unknown. We found that HFD fed mice showed higher mRNA expression of ICAM-1 and VCAM-1 in adipose tissue. Previous studies also reported that expression of adhesion molecules in adipose tissue was increased by obesity (26, 27). In addition, obese mice did not exhibit macrophage infiltration into adipose tissue after ICAM-1 antagonist treatment (28). Thus, adhesion molecules affect obesity-induced macrophage infiltration into adipose tissue. However, the effect of exercise training on ICAM-1 expression in adipose tissue has been unclear until now. Interestingly, we observed that ICAM-1, but not VCAM-1, was significantly reduced by exercise training. Another study also showed that plasma ICAM-1 concentrations were markedly decreased by moderate exercise training in type II diabetic patients without a concomitant weight loss (29). Although it needs more study to clearly establish in detail for the effect of exercise training on ICAM-1 in adipose tissue, attenuated ICAM-1 might be one of the causes of exercise training-induced suppression of macrophage infiltration. On the other hand, it is known that mice who are deficient for the macrophage specific chemokines, such as MCP-1 and

CXCL14, do not exhibit inflammation despite their obesity (9, 12). However, in this study, inhibitory effects of exercise training on the expression of MCP-1 and CXCL14 in adipose tissue were not observed, although we clearly found that HFD fed mice showed high expression of those chemokines in adipose tissue. Taken together, our data indicate that exercise training-induced down-regulation of pro-inflammatory cytokine mRNA expression is caused by depression of macrophage infiltration into adipose tissue in HFD-induced obese mice.

Our study, however, revealed another possible mechanism for the exercise training-induced decrease in pro-inflammatory cytokine expression. Down-regulation of CD11c mRNA, reflecting the presence of M1 macrophage, and up-regulation of CD163 mRNA, reflecting the presence of M2 macrophage, in adipose tissue were observed in HFD exercised mice. Thus, our data indicated that exercise training induced macrophage phenotypic switching from M1 to M2 in adipose tissue. We speculate that moderate exercise might inhibit M1 macrophage infiltration into adipose tissue. Indeed, infiltrated macrophages into adipose tissue in obesity are mainly M1 macrophages. Patsouris et al. (14) showed that a treatment of CD11c antagonist attenuated inflammation via inhibition of M1 macrophage infiltration in obese mice. These studies suggest that the phenotypic switch of M1/M2 macrophages causes obesity-induced pro-inflammatory cytokine production in adipose tissue. In this study, we also observed both a phenotypic switch of M1/M2 macrophages and obesity-induced pro-inflammatory cytokine production in adipose tissue. Moreover, exercise training in these obese mice not only induced inhibition of the M1 macrophage marker but also increased the M2 macrophage marker, while the exercise attenuated higher TNF-α mRNA expression in adipose tissue. In fact, the M1/M2 ratio in HFD-treated mice was greatly inhibited by exercise training (HFD control vs. HFD exercise: 57% vs. 11%), although the ratio in ND treated mice was 1.1% in both control and exercised animals. However, it is difficult to explain why down-regulation of CD163 mRNA occured in our study. Another possibility is that exercise training induces differentiation from M1 to M2 macrophages in adipose tissue. This hypothesis was based on data that glucocorticoid treatment interfered with the increase in M1 macrophages in adipose tissue, and inhibited inflammation in obese mice (30) and moderate exercise induces glucocorticoid (31). In addition, a recent study reported that monocytes up-regulated CD163 after treatment with dexamethasone (32). We also observed that dexamethasone treatment induced a decrease in CD11c mRNA expression and an increase in CD163 mRNA expression *in vitro* (data not shown). Therefore, moderate exercise-induced glucocorticoid secretion may affect macrophage differentiation from M1 to M2.

Glucocorticoid inhibits the expression of TLR2 and TLR4 (33). It is possible that the attenuation of TLR4 expression in exercised mice was caused by exercise-induced glucocorticoid stimulation. Furthermore, an exercise training-induced reduction in TNF-α mRNA expression might be regulated by the down-regulation of TLR4. Activation of TLR4 with free fatty acids stimulates NF-κB signaling and expression of TNF-α and IL-6 in adipocytes (22, 23). Therefore, our results suggest that exercise-induced down-regulation of TLR4 expression, which is occurred by both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in adipose tissue,

attenuates pro-inflammatory cytokine expression in adipose tissue of high-fat-diet-induced obese mice.

The limitation of this study was that the experiment was carried out without isolation of macrophages from adipose tissue. Several parameters, such as TNF-α, MCP-1 and TLR4, are expressing on/in adipocytes as well as macrophages. However, macrophages, but neither adipocytes nor other cells, in mice specifically express F4/80. In addition, expression of CD11c and CD163 has never been observed in adipocytes in previous studies, although future studies need to study using an analysis of isolated macrophage functions from adipose tissue. Another limitation was that the evaluation of protein levels in each parameter was not carried out. Altough mRNA expression levels are commonly used as a proxy for estimating functional differences that occur at the protein level, future investigations need to identify exercise-induced CD11c and CD163 mRNA expressions as the phenotypic switch of macrophage polarization in adipose tissue.

CONCLUSION

Exercise training is now considered to be a crucial event leading to a reduction of inflammation in adipose tissue. However, it has still been unclear how exercise training down-regulates the inflammatory states of adipose tissue. The present study demonstrates that exercise training markedly inhibits TNF-α and F4/80 mRNA expression in adipose tissue of obese mice. Furthermore, both decreased CD11c mRNA and increased CD163 mRNA expression in adipose tissue were observed with the exercise training. Also, the higher mRNA expression of TLR4 was reduced by the exercise training. These results suggest that exercise training might induce the phenotypic switching from M1 to M2 macrophages in adipose tissue as well as inhibiting M1 macrophage infiltration into adipose tissue. Therefore, chronic exercise might contribute to inhibition of inflammation in adipose tissue via down regulation of TLR4.

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