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The Differential Impact Of High-Intensity Swimming Exercise and Inflammatory Bowel Disease On IL-1β, TNF-α, and COX-2 Gene Expression in the Small Intestine and Colon in Mice

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ABSTRACT

Background and Objective

We aimed to examine the impact of high-intensity swimming exercise and inflammatory bowel disease (IBD) on IL-1β, TNF-α, and COX-2 gene expression in the small intestine and colon of mice.

Material and Methods

Forty male C57BL/6 mice were divided into 4 groups: the control group (CON), swimming exercise group (EX), 50% ethanol (EtoH) control group (50%EtoH CON), and 2,4,6-trinitrobenzene sulfonic acid group (TNBS). The EX group performed 4 weeks of exercise. Intrarectal TNBS injection induced IBD in the TNBS group; the 50%EtoH CON group received control injections. Reverse transcription and real-time polymerase chain reaction were used to examine IL-1β, TNF-α, and COX-2 mRNA expression in the small intestine and colon.

Results

IL-1β, TNF-α, and COX-2 mRNA expression was significantly increased in the EX group compared to that in the CON group (p's<0.05). IL-1β and COX-2 mRNA expression was significantly increased in the TNBS group compared to that in the 50%EtoH CON group (p's<0.05).

Conclusion

Thus, inflammatory cytokine IL-1β and COX-2 expression in the small intestine and colon was increased in both high-intensity swimming exercise and IBD models. However, TNF-α was increased only in the swimming exercise model. Further research is required to confirm these observations and establish swimming exercise regimes appropriate for patients with IBD.

Recently, the incidence of body disease related to a lack of exercise or over-exercise has rapidly increased. Many studies have focused on exercise for the prevention of disease; however, contrasting results are receiving attention, which suggest that over-exercise, such as high-intensity exercise, increases the risk of infection from disease and inflammation through an imbalance in the immune system.[1,2] Exercise induces inflammatory cytokines IL-1β and TNF-α, stimulating T and B cell production through inflammation induction and immune control action.[3] Thus, high-intensity exercise can be a cause of diseases related to abnormalities in the immune system via the induction of inflammatory cytokines.[4] Furthermore, high-intensity exercise causes inflammation through ischemia to the organs. In particular, exercises such as running a marathon can lead to various diseases through an imbalance in the immune system caused by ischemia to the digestive organs.[5] Ischemia to the digestive system can cause stomach-aches, diarrhea, bleeding, and inflammation within the intestine.[6] However, other studies have reported that exercise can restrict obesity and inflammation-related gene expression in the intestine.[7] In addition, it has been reported that exercise can prevent many diseases of the large intestine.[8] However, with the exception of the few studies mentioned above, there is generally a lack of studies examining the correlation between exercise and immune activity in the large intestine.

Recently, the incidence of diseases of the large intestine, such as inflammatory bowel disease (IBD) and ulcerative intestinal disease, have been increasing.[9] IBD is an autoimmune disease. Although no known causes of IBD have been revealed, genetic, immune, and environmental factors are thought to be involved.[10] IBD involves chronic recurrent inflammation that invades the digestive canal from the mouth to the anus. IBD is classified as ulcerative colitis when continuous lesions in the mucous and submucous layers exist, and as Crohn's disease when the mucous membrane to the mucous layer is affected.[11] In a clinical trial, impaired mucosal barrier function and cytokine generation were included as causes of IBD[3]; however, evidence regarding the role of cytokines with regard to IBD pathogenesis remains unclear. Recently, numerous studies on proinflammatory cytokine generation have reported that the generation of IL-1β and TNF-α around the inflammatory area is rapidly increased in patients with IBD. Studies regarding cytokine generation and control are critical in understanding the physiologic mechanisms underlying IBD.[12] The increase in the expression of representative inflammatory cytokines in IBD (such as IL-1β and TNF-α) may be the main cause of inflammatory cell activation.[13,14] On the other hand, COX-2 is an early immune gene expressed in inflammatory cells (such as macrophages) during the stimulation of inflammation and cytokine production, but is not expressed during normal conditions.[15]

Thus, the genes that encode inflammatory factors in the digestive system, which damage and cause inflammation in the tissue after vigorous physical exercise, are similar to those in the acute phase response to inflammation by infection and those involved in IBD. Animal studies that examine the impact of IBD on the large intestine are necessary, as limitations exist in measuring bowel movement function or conducting epidemiologic and factual studies in patients with IBD.[16] Moreover, animal studies are critical for examining differences in the impact of high-intensity exercise and the induction of IBD on immune system activity in the intestine.

The aim of the present study was to investigate the differential impact of a high-intensity swimming exercise and induced IBD on immune activity in the digestive system using a mouse model.

METHODS

Experimental Animals and Dietary Methods

After environmental adaptation, 40 male C57BL/6 mice (8 weeks old; 20 g) were randomly assigned into 4 groups of 10 mice each: the control group (CON), swimming exercise group (EX), 50% ethanol (EtoH) control group (50%EtoH CON), and 2,4,6-trinitrobenzene sulfonic acid group (TNBS). The experiment was conducted at a vivarium in the Gwangju Institute of Science and Technology, which breeds experimental animals. Commercially-available Samtako mouse food (protein, 22.5%; fat, 3.5%; low-fibre, 7.0%; potassium, 9.0%; calcium, 0.7%; phosphorus, 0.5%) and water were adequately supplied. The on-off light

cycle was set to 12 hours, temperature was set to 22±2°C, and humidity was set to 70~80%. All study procedures were approved by the ethical review board at the Gwangju Institute of Science and Technology.

Experimental Design and Exercise Methods

Exercise experiment. The EX group performed a high-intensity swimming exercise, 5 times a week for 4 weeks. The swimming exercise was conducted in a plastic swimming pool 60 cm in height and 120 cm in diameter. The water temperature of the swimming pool was set to 35–37˚C. In order to not allow weight-bearing during swimming, the pool was filled with water to at least 45 cm. During the first week, the swimming exercise was performed for 30 minutes; 4% of the body weight was added to the tail starting in the second week. During the 3rd week, the swimming exercise was performed for 60 minutes with 7% of the body weight added to the tail. Small intestine and colon tissues were extracted after 24 hours of exercise in the 4th week and immediately placed in a −80°C liquid nitrogen tank. Small intestine and colon tissues were similarly extracted from the CON group, which did not perform the exercise and served as a control for the EX group.

IBD experiment. The 50%EtoH CON group, which served as the control for the TNBS group, was intrarectally injected with 100 μl of ethanol, while the TNBS group was intrarectally injected with 100 μl of 4% TNBS (TNBS, 4%/100 μL, 50% ethanol). Twenty-four hours after injection, small intestine and colon tissues were extracted for analysis.

Analysis Method

RNA isolation. Tissue samples less than 50 mg were extracted from the large and small intestine using surgical instruments without RNAse. Trizol reagent (1 mL; JBI, Korea) was added to the tissue samples in a 1.5-mL Eppendorf tube, pulverized, and centrifuged for 15 minutes at 20 seconds of vortex, 4˚C, and 15,000 rpm, after adding chloroform. At this time, isopropyl alcohol was added; after 7 minutes at room temperature, centrifugation at 15,000 rpm and 4˚C was performed for 20 minutes. The sample was washed with 75% EtoH in diethyl pyrocarbonate (DEPC) water after removing the supernatant, and was centrifuged at 13,000 rpm and 4˚C for 5 minutes, followed by removal of the supernatant and DEPC water-melted pellet. The RNA extracted from this process was dissolved in 0.1% DEPC water and the absorbance was measured at 260 nm/280 nm using a UV spectrophotometer. The RNA quality was measured in 5 mice from each group, including the 5 mice that best completed the exercise condition. RNA from 3 mice in each group with good RNA quality was further examined using reverse transcription and real-time polymerase chain reaction (PCR).

Reverse Transcription- PCR (RT-PCR). mRNA expression in the large intestine was quantified using the semi-quantitative RT-PCR method. In the reverse transcription reaction, the total extracted RNA (1 μg) and Oligonucleotide dT primer (100 pmol) were mixed in an Accupower® RT PreMix (Bioneer Co, Korea) tube and pre-processed for 5 minutes at 70°C, with a total reaction amount of 20 μl. cDNA synthesis was performed at 42°C for 1 hour, and a heat treatment was conducted at 80°C for 15 minutes to deactivate the reverse transcriptase. The PCR was polymerized using reversely transcribed cDNA (1 μg) and Forward and Reverse Primers (10 pmol) in an Accupower® PCR PreMix (Bioneer Co., Korea) tube. After denaturing the cDNA with a heat treatment at 94°C for 5 minutes, polymerization was conducted as follows. TNF-α was polymerized by 5′-GGC AGG TCT ACT TTG GAG TCA TTG C-3′ (sense) and 5′-ACA TTC GAG GCT CCA GTG AAT TCG G-3′ (antisense); COX-2 was polymerized by 5′-GAA GGG ACA CCC TTT CAC AT-3′ (sense), 5′-ACA CTC TAT CAC TGG CAT CC-3′ (antisense); and GAPDH was polymerized by 5’-AAT GCA TCC TGC ACC ACC AA-3’ (sense) and 5’-GTA GCC ATA TTC ATT GTC ATA-3 (antisense). The number of PCR cycles was 28, as suggested.

Real-time PCR. Real-time PCR involved amplification using a DyNAmo SYBR Green qPCR kit and DNA Engine Opticon (MJ Research, USA). cDNA mold and each specific primer were added to a 2X PCR master mix and adjusted to the PCR condition before the reaction was conducted. After quantifying against GAPDH expression, the degree of increase in gene expression (treatment group vs control group) was calculated using the following equation: fold change=2-ΔΔCT; ΔΔCT= (CT, Target - CT, GAPDH) at time x - (CT, Target - CT, GAPDH) at time 0, where

time x is a random time and time 0 is the moment at which the gene expression of interest, quantified against GAPDH expression, becomes 1 in the control group.

Statistical Analysis

Analyses were performed using SPSS ver. 20.0 (IBM Corp., Armonk, NY, USA). All results are expressed as mean ± standard deviation. A Kolmogorov-Smirnov sample test was conducted to evaluate the distribution, as the number of subjects was small; no issues were found. Independent t-tests were conducted to examine group differences. A $p<0.05$ was considered statistically significant.

RESULTS

The Impact of Exercise on MRNA Expression in Small and Large Intestine Tissue

In the RT-PCR analysis of the small and large intestine, the EX group showed significantly increased IL-1β, TNF-α, and COX-2 mRNA expression compared to that in the CON group ($p<0.05$) (Figure 1A). Similarly, in the real-time PCR analysis of large and small intestine, the EX group showed significantly increased IL-1β, TNF-α, and COX-2 mRNA expression compared to that in the CON group ($p<0.05$) (Figure 1B, C, and D).

MRNA EXPRESSION IN SMALL AND LARGE INTESTINE TISSUE IN AN IBD MODEL

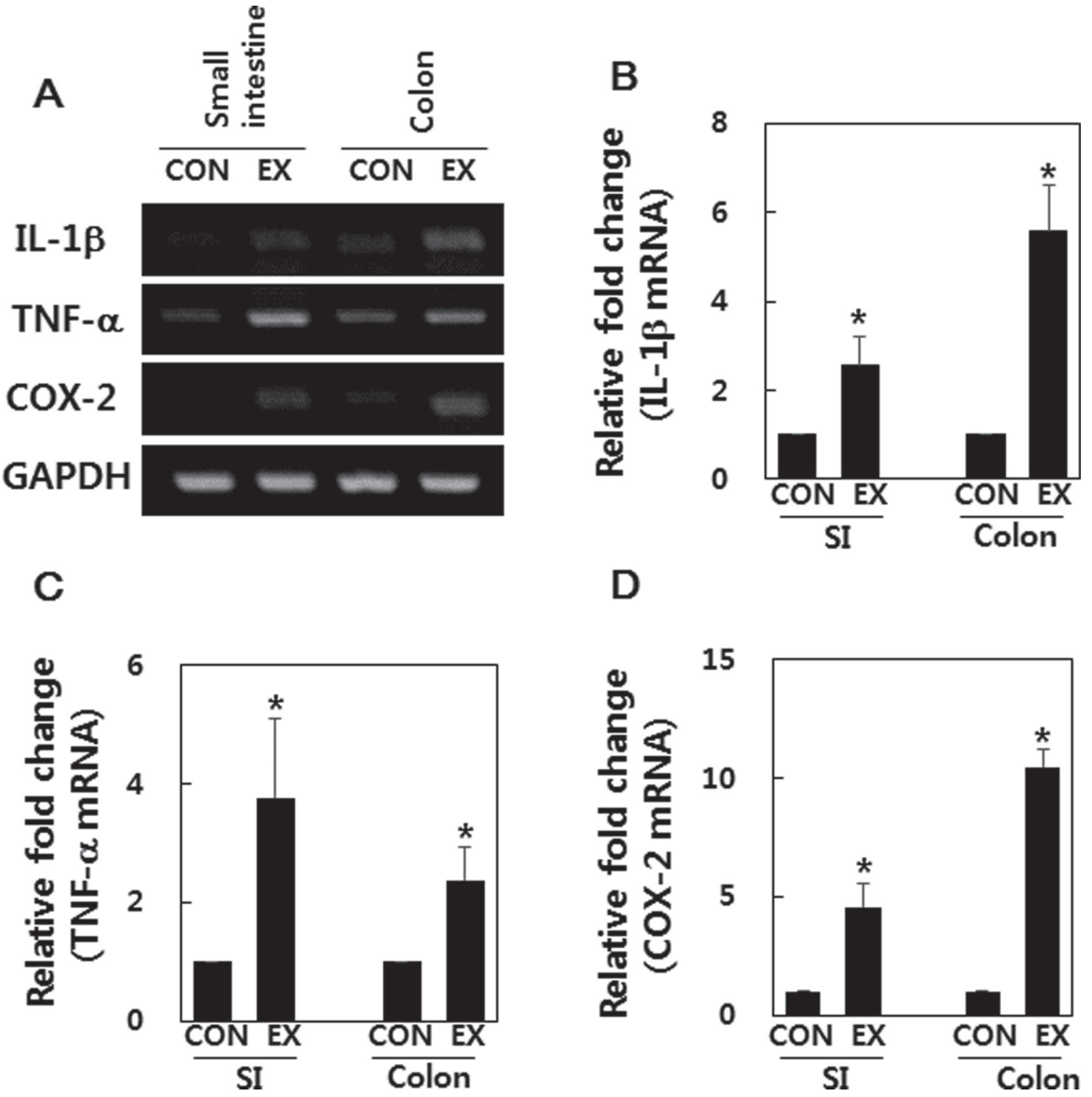
In the RT-PCR analysis of the large and small intestine in an IBD model, the TNBS group showed significantly increased IL-1β and COX-2 mRNA expression compared to that in the 50%EtoH CON group ($p<0.05$). However, the groups did not differ in TNF-α expression ($p>0.05$) (Figure 2A). Similarly, in the real-time PCR analysis of the large and small intestine in an IBD model, the TNBS group showed significantly increased IL-1β and COX-2 mRNA expression compared to that in the 50%EtoH CON group ($p<0.05$). However, the groups did not differ in TNF-α expression ($p>0.05$) (Figure 2B, C and D).

DISCUSSION

In the present study, we investigated the differential impact of a high-intensity swimming exercise and IBD induction on immune activity in the digestive system using inflammatory markers. Several recent studies have examined the relationship between exercise and large intestine disease.[17,18] In one report, differences in exercise intensity were shown to affect IBD-related gene expression in the large intestine.[19] This result suggests that exercise type and intensity can create a similar condition to that in IBD within the intestine. However, other studies have demonstrated that exercise can specifically increase anti-inflammatory and anti-immune cell production in the digestive system, preventing most inflammatory diseases.[8] In the present study, we examined the expression of IL-1β, TNF-α, and COX-2, which are inflammatory markers of the immune system, in a swimming exercise model and an IBD model. In IBD, cytokines play an important factor in inflammation and the immune reaction; moreover, cytokines play an important role in the immune system in the intestinal tract.[20] In addition, exercise is known to induce inflammatory cytokines;[21] however, there is lack of studies regarding whether exercise can induce inflammatory cytokines in the intestine. Overall, there is evidence that both high-intensity exercise and IBD can act as stressors in the intestine.

In the present study, a high-intensity swimming exercise was shown to increase IL-1β, TNF-α, and COX-2 mRNA expression in the intestine. Thus, increases in inflammation-related factors following a swimming exercise are similar to the environment in the large and small intestine following a high-intensity swimming exercise. However, an increase in HO-1 (an anti-inflammatory gene) during high-intensity exercise can decrease organ damage caused by shock in intestinal tissue and can protect cells from the toxic effects of cytokines.[22] Exercise both increases and decreases the expression of the inflammatory cytokine, TNF-α.[23] Exhaustive or high-intensity exercise has been shown to temporarily increase inflammatory cytokines such as IL-1β and TNF-α.[24,25] Furthermore, a possibility exists that exercise-induced oxidative stress caused by high-intensity exercise increases the formation of harmful oxygen and directly stimulates monocytes and macrophages, resulting in acute reactions such as that of anti-inflammatory cytokines.[26] These results are in line with the results of the present study. However, future multilateral studies, in contrast to

FIG. 1 IL-1β, TNF-α, and COX-2 gene expression in a high-intensity swimming exercise group (EX) and control group (CON), as determined by reverse transcription polymerase chain reaction (PCR) (A) and real-time PCR (B, C and D).

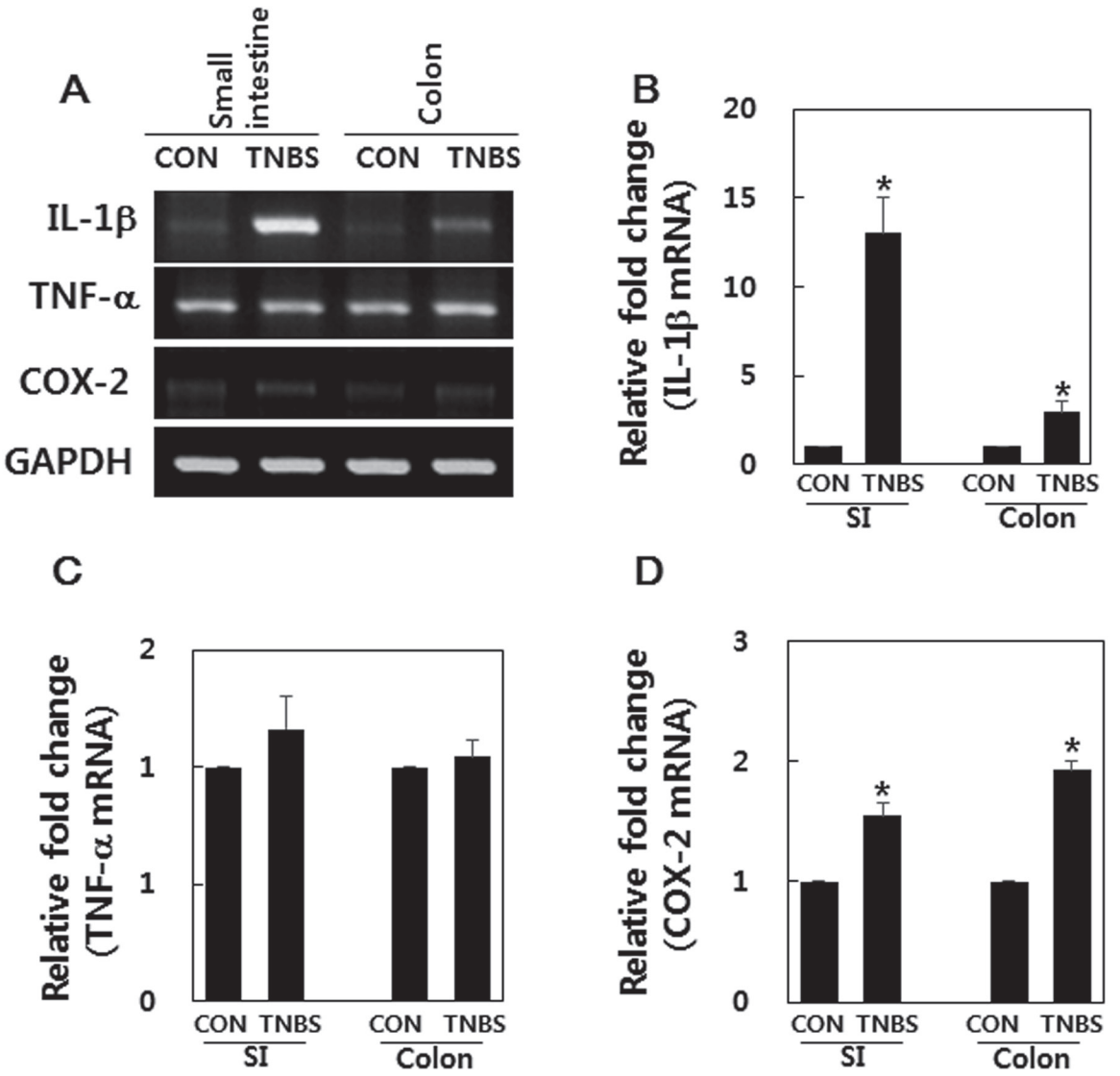


CON = control group, EX = swimming exercise group, SI = small intestine.
**Significantly different compared to the control group at p<0.05.*

the current unilateral study, should be conducted, as inflammatory and anti-inflammatory genes have shown opposing results.

IBD, known as Crohn's disease or ulcerative colitis, is an ill-defined disease with chronic inflammation and corresponding complications. Although the cause of IBD is not yet defined, immune factors such as immune-mediated tissue damage, genetic factors, smoking, diet, environment, and microbiological factors, such as flora, are thought to be complexly

FIG. 2 IL-1β, TNF-α, and COX-2 gene expression in the induced inflammatory bowel disease group (TNBS) and control group (CON) as determined by reverse transcription polymerase chain reaction (PCR) (A) and real-time PCR (B, C and D).



CON = control group, TNBS = induced inflammatory bowel disease, SI = small intestine.
**Significantly different compared to the control group at p<0.05.*

involved.[27] Especially, inflammatory markers such as cytokines can induce tissue damage leading to uncontrolled immune and inflammation reactions, resulting in chronic inflammation.[28]

In the present study, IL-1β and COX-2 mRNA expression in the small and large intestine was significantly increased in an IBD-induction group compared to expression levels in a control group; however, TNF-α expression was not significantly increased. IL-1β is involved in IBD occurrence; however, the mechanism remains unknown.[29] IL-1β and COX-2 expression has been shown to increase following an injection of

rumen microorganisms, which are known to play an important role in IBD.[30] The present results suggest that inflammatory cytokines are increased by IBD. However, a previous study showed that TNBS-induced mice had decreased inflammatory cytokine expression (IL-1β and TNF-α) after 6 weeks of wheel running.[3] Although the results of this previous study suggest that physical activity has a positive effect on IBD, high-intensity exercise can induce a genetic environment similar to IBD, as suggested in the present study.

The results of the present study caution against engaging in high-intensity exercise and suggest that appropriate physical exercise regimes must be established for patients with IBD.

CONCLUSION

The present study investigated differences in inflammatory cytokine profiles in a swimming exercise model and an IBD model using C57BL/6 mice. In the swimming exercise and IBD models, inflammatory cytokine IL-1β and COX-2 expression was increased. However, the swimming exercise model, but not the IBD model, showed increased TNF-α expression.

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The authors have no conflicts of interest to declare.

DISCLOSURE

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