Effects of Exercise Intensity on Alcohol Dehydrogenase Gene Expression in the Rat Large Intestine

Exercise and ADH Gene Expression
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ABSTRACT

Background and Objective
The aim of this study was to examine the relationship between the intensity of treadmill exercise and the expression of alcohol dehydrogenase (ADH) in the large intestine.

Materials and Methods
Thirty Sprague-Dawley male rats were randomly assigned to a control group (CON; no exercise), low-intensity exercise group (LIG; 30-min exercise at 8 m/min 5 times a week for 4 weeks), and high-intensity exercise group (HIG; 30-min exercise at 28 m/min 5 times a week for 4 weeks).

Results
Microarray analysis was conducted to evaluate ADH gene expression levels in large intestinal tissue. Significant changes in the expression of four ADH genes (Adh1, Adh4, Adh6a, and Adh7) were observed in relation to exercise intensity. In addition, pooled samples in the exercise groups showed decreased expression levels of these four genes compared with those of the control group. These findings were confirmed by reverse transcription-polymerase chain reaction. In addition, differences were detected with respect to exercise intensity; Adh1, Adh4, and Adh6a levels were significantly decreased in the LIG compared with those in the HIG, whereas Adh7 expression showed an opposite trend.

Conclusion
In conclusion, this study suggested that regular exercise decreases the incidence of alcohol-related disease by suppressing ADH production in the digestive system.
Genomic analyses have become more common in the sports medicine field with regard to exercise-induced changes in molecular and cell biological events as well as the development and adaption of the immune system.\textsuperscript{1} In particular, such research has rapidly increased since the completion of the genome maps of humans and several important model animals. Previous genome studies in sports medicine have mostly focused on the bodily and cellular responses to exercise. However, since it is now possible to analyze the responses of tens of thousands of genes simultaneously, it is possible to rapidly determine the expression profiles and functions of various proteins.\textsuperscript{2} Microarray analysis is a cutting-edge method to analyze the whole genome and has enabled detailed analyses related to disease diagnosis and genetic information.\textsuperscript{3} Namely, microarray profile studies using DNA chips could contribute to the development of guidelines for exercise based on data collected from various body tissues.\textsuperscript{4}

The incidence of diseases of the large intestine has increased in recent years owing to poor eating habits combined with physical inactivity, which has motivated substantial research of strategies for large intestine disease prevention.\textsuperscript{5} Exercise directly and indirectly affects the large intestine,\textsuperscript{6} and sufficient physical activity can help reduce the risk of large intestine disease.\textsuperscript{7} However, the specific mechanisms through which genes interact with exercise to exert positive effects on digestive metabolism remain unknown.\textsuperscript{8} The large intestine is affected by both exercise and dietary habits. In particular, alcohol consumption directly affects every organ in the body, including the digestive system.\textsuperscript{9} Excessive alcohol consumption contributes to stomach pain, diarrhea, and bloody excrement, but exercise has recently been highlighted as a potential strategy to alleviate the negative effects of alcohol on the body.\textsuperscript{10} Despite some reports showing an effect of exercise on alcohol metabolism, few genomic studies have examined the underlying mechanisms driving these effects.\textsuperscript{11}

Alcohol is mainly processed by the oxidative system. Alcohol that is absorbed in the body is usually metabolized to acetaldehyde by alcohol dehydrogenase (ADH) in the cytoplasm.\textsuperscript{12} Although activation of ADH in the digestive system is lower than that in the liver, it nevertheless affects the bioavailability of alcohol.\textsuperscript{13} Subsequently, acetaldehyde is decomposed to acetic acid by aldehyde dehydrogenase in the mitochondria, which is essential for proper alcohol metabolism, as it acts in the common final metabolic process of alcohol.\textsuperscript{14} Accumulation of acetaldehyde, the primary product of alcohol metabolism, has a toxic effect on various parts of the body, leading to flush and vomiting; moreover, acetaldehyde contributes to alcohol abuse/excessive consumption behaviour.\textsuperscript{15} Although substantial research efforts have been made to understand the body’s response in the context of acetaldehyde accumulation after alcohol consumption,\textsuperscript{16} the mechanisms underlying the roles of ADH in ethanol metabolism and their associations with exercise remain poorly understood.

Given the recent developments in reliable molecular genetic methods to study the relationships between disease and gene expression, the aim of the present study was to evaluate whether differences in exercise intensity affected ADH gene expression in the large intestine through microarray profiling using a rat model.

**METHODS**

**Animals**

Thirty 8-week-old, Sprague-Dawley white male rats (200 ± 15 g) were randomly selected for the study. The rats were equally divided into three groups: the control (CON) group, low-intensity exercise group (LIG), and high-intensity exercise group (HIG).

**Experimental Design and Exercise Method**

The CON group did not perform any exercise-related activity, whereas the two exercise groups performed 10–15 min of treadmill running at 5–20 m/min for 1 week before the start of experiments, as adaptation. After 1 week of adaptive exercise, rats in the LIG performed 40–45\% VO\textsubscript{2max} treadmill exercise at 8 m/min 5 times per week for 4 weeks with 30-min exercise bouts, and rats in the HIG performed 75\% VO\textsubscript{2max} treadmill exercise at 28 m/min 5 times per week for 4 weeks with 30-min exercise bouts.\textsuperscript{17} Within 48 h after the last treadmill exercise, cervical vertebral dislocation was conducted in all experimental animals to eliminate the immediate effects of exercise, and large intestine tissue was extracted and immediately stored in a liquid nitrogen tank at −80°C.
**Exercise and ADH Gene Expression**

**RNA Isolation**

Large intestinal tissues (< 30 mg) were placed in a 1.5-mL Eppendorf tube without RNase. RNA was extracted with 1 mL TRIzol reagent (JBI, Seoul, Korea). The tissue was pulverized, chloroform was added to the tube, and the mixture was centrifuged at 15,000 rpm at 4°C for 30 min. After the addition of isopropanol, the sample was kept at room temperature for 7 min and then centrifuged at 15,000 rpm at 4°C for 20 min. The obtained pellet was washed with 75% ethanol in diethyl pyrocarbonate (DEPC) water and centrifuged at 13,000 rpm and 4°C for 10 min, and the supernatant was then discarded to obtain a pellet suspended in DEPC water. The extracted RNA was dissolved in 0.1% DEPC water, and the absorbance was measured at 260/280 nm using an ultraviolet spectrophotometer to determine RNA quality. The five best-performing rats that completed the resistance exercise were selected from the 10 rats per group for subsequent analysis. After quality evaluation, the RNA samples from three rats with the highest-quality RNA were used for real-time polymerase chain reaction (PCR) analysis.

**Microarray Analysis**

A microarray analysis was conducted using the 60-mer AB Rat Genome Survey microarray system with 33,096 oligonucleotide probes targeting a total of 26,857 rat genes. cRNA labelled with digoxigenin-UTP was processed according to protocol P/N 4339629 and applied to the Applied Biosystems Chemiluminescent RT-IVT Labelling kit v2.0 (P/N 4340472) using 2 μg of total RNA. Images were quantified according to detection of the chemiluminescent signal and background of the automated grid and then standardized using Applied Biosystems 1700 Chemiluminescent Microarray Analyzer software v. 1.1.1.

**Reverse Transcription (RT)-PCR**

PCR products were quantified to determine relative mRNA levels by semiquantitative RT-PCR for the four genes found to be differentially expressed in large intestinal tissues based on the microarray results. For the RT reaction, the sample was preprocessed at 70°C for 5 min in a total volume of 20 μL after mixing total RNA (1 μg) with oligonucleotide dT primer (100 pmol) and Accupower RT PreMix (Bioneer Co., Seoul, Korea) in a tube. cDNA synthesis was conducted at 42°C for 1 h, followed by heat treatment at 80°C for 15 min to deactivate the reverse transcriptase. PCR was conducted using 1 μg of the reverse-transcribed cDNA and 10 pmol of each forward and reverse primer targeting Adh1, Adh4, Adh6α, and Adh7 in an Accupower PCR PreMix (Bioneer Co.) tube.

**Statistical Analysis**

To analyze the microarray data of large intestinal tissues according to differences in exercise intensity, the data were normalized to reveal systematic variations in the effects of exercise on gene expression levels. Internal normalization was applied to determine changes based on the remaining data for image analysis. The data were visualized by matrix plot analysis. Graphs were constructed for 32 gene expression levels from the CON, LIG, and HIG. This analysis was performed to determine the significance of differences in gene expression levels among groups with reference to the level of the control group, when the ratio of the two groups (CON/CON) was 1. To determine the pairwise differences between groups for the four genes showing significantly different expression among the groups in the microarray analysis (Adh1, Adh4, Adh6α, and Adh7), the mRNA was pooled for 10 rats per group. Statistical analysis of the microarray data was performed using AVADIS prophetic version 3.3 (Strand Genomics, Bangalore, India) software. Statistical significance was set at $p < 0.05$.

**RESULTS**

A microarray analysis using RNA isolated from the large intestine of rats from the three groups revealed significant differences in the expression levels of four ADH genes (Adh1, Adh4, Adh6α, and Adh7) in relation to exercise. When the mRNAs of the 10 rats in each group were pooled, a microarray analysis showed that the expression levels of Adh1, Adh4, Adh6α, and Adh7 were significantly decreased in the LIG and HIG compared with those in the CON group. Furthermore, in the exercise groups, Adh1, Adh4, and Adh6α expression levels were significantly decreased in the LIG compared with those in the HIG, whereas Adh7 levels were significantly decreased in the HIG compared with those in the LIG (Table 1). RT-PCR
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Analysis further confirmed that the mRNA expression levels of Adh1, Adh4, Adh6a, and Adh7 were significantly decreased in the LIG and HIG compared with those in the CON group (Figure 1A and B).

DISCUSSION

We applied recently developed DNA chip microarray technology to determine gene expression patterns in the large intestine of rats according to differences in exercise intensity. Specifically, the differences in ADH gene expression in the large intestine in each exercise group (LIG and HIG) was determined relative to that in the nonexercise group (CON), using 4 weeks of exercise. The results demonstrated that Adh1, Adh4, Adh6a, and Adh7 expression levels were significantly decreased by exercise.

ADH genes have been reported to be directly and indirectly associated with exercise, although there is only partial knowledge on alcohol metabolism by ADH in various organs of white rats. ADH is distributed throughout the digestive system, and reduction in ADH gene activity and consequent enzyme activity results in the accumulation of aldehyde, which increases the risk of digestive system disease. Moreover, the ADH gene is known to be related to alcohol as well as dosage, and alcohol metabolism-related gene expression and cancer incidence are related to ADH and aldehyde dehydrogenase. Additionally, individuals with genetically higher activation of ADH have higher levels of acetaldehyde, the primary product of alcohol that can cause digestive system cancers.

A previous study showed that ADH1 expression was upregulated by ethanol administration but was downregulated upon administration of antioxidative drugs. In addition, the decrease in ADH expression by exercise has been shown to increase the bioavailability of alcohol in humans. Exercise can also alleviate the side effects of alcoholism because it can act as an ADH enzyme inhibitor. Moreover, exercise may decrease the detrimental effects of alcohol by decreasing the risk of digestive system disease.

<table>
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<tr>
<th>TABLE 1 Microarray Analysis of Alcohol Dehydrogenase Gene Expression</th>
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<tr>
<td><strong>Gene Name</strong></td>
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<tr>
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<tr>
<td>Alcohol dehydrogenase 1</td>
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<td>Alcohol dehydrogenase 4</td>
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<tr>
<td>Alcohol dehydrogenase 6A</td>
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<td>Alcohol dehydrogenase 7</td>
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CON = control group; LIG = low-intensity exercise group; HIG = high-intensity exercise group.

FIG. 1 Adh1 and Adh4 (A), and Adh6A and Adh7 (B) mRNA expression levels related to exercise intensity, as determined by RT-PCR. CON, control group; LIG, low-intensity exercise group; HIG, high-intensity exercise group; GAPDH expression was used as an internal control.
altering the fiber area of tissues and decreasing capillary density. Our findings suggested that exercise could reduce the maximum level of rapidly produced aldehyde by inhibiting ADH gene and enzyme expression. However, additional detailed studies are required to determine the specific mechanisms linking exercise and ADH gene expression, particularly with respect to the four genes found to be differentially expressed.

Our results confirmed that exercise influenced the expression of ADH in the large intestine, which is partly related to the intensity of exercise performed. Further investigation of the underlying mechanisms and confirmation in humans in the context of alcoholism could help to establish an exercise regimen as part of standard treatment or prevention of alcohol-related digestive diseases.

CONCLUSION

Regular exercise decreased the risk of alcohol-dependent disease by inhibiting ADH gene expression and enzyme activity in the digestive system. This work could serve as a useful reference for further studies on the effects of exercise on alcohol metabolism in the digestive system. With regard to clinical applications, these findings provide insights into our understanding of the genetic environment involved in alcohol metabolism in the intestine and may facilitate studies of the mechanism of ADH related to exercise. Based on these basic studies, we believe that exercise may have positive effects on the intestinal environment by controlling intestinal ADH. Moreover, exercise may significantly affect alcohol bioavailability in the digestive system and control alcohol metabolism.

ACKNOWLEDGEMENTS

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REFERENCES