Differences of Wood Vinegar Ingestion and Exercise Training on Blood Lipids, MDA, and SOD Activities in Rats

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The purpose of this study is to identify the effects of exercise training and oak tree wood vinegar ingestion on the blood lipids and antioxidant activities of rats. The subjects were 28 Sprague Dawley male rats, and they were assigned into four groups (n=7, respectively): the control group (CON), the exercise group (EXE), the vinegar ingestion group (VIN), and the vinegar ingestion and exercise training group (VINEXE). The diet was based on high fat and oral administration of oak tree wood vinegar. The rats that were not given oak tree wood vinegar were given the same amount of distilled water orally in order to maintain the same level of stress. They were exercise trained on motor-driven treadmills during a four-week session. Weight changes in the VINEXE were significantly inhibited in the later period of exercise, when compared to the CON (p<0.05). Fat increase was significantly suppressed in VIN and EXE (p<0.05), and a synergistic effect was discovered in the VINEXE (p<0.05). Glucose and ammonia levels were significantly reduced in the EXE, VIN, and VINEXE compared to the CON (p<0.05). In blood lipids, TC and LDL-C were significantly enhanced in the EXE, VIN, and VINEXE compared to the CON (p<0.05), while HDL-C was significantly improved in the EXE and VINEXE (p<0.05). Liver MDA contents showed significant changes in each group (p<0.05), and SOD activities were significantly enhanced in the VIN and the VINEXE when compared to other groups (p<0.05). Therefore, oak tree wood vinegar ingestion with exercise training for four weeks may result in inhibition of weight gain, improvement of blood lipids, and inhibition of lipid peroxidation, contributing to health promotion.

Key words: Wood vinegar, blood lipids, SOD, MDA, exercise training

Introduction

With recent interests in health, the efficiencies of charcoal have been approved trough various media, and utilized widely in everyday life. Charcoal was used only as a kind of fuel for roasting meat but nowadays is being proved to have detoxication, immunity, antibacterial activity, and purification activity through scientific research.

Recently, anti-oxidants are developed by using various natural substances, and researchers tend to focus on rather natural antioxidant substances including Sanghwang mushrooms [23], black beans [19], and oak tree wood vinegar [3] than ordinary food. Many researchers prefer natural substances because some synthetic substances are under strict regulations in their amount in that parts of the substances are possible to be toxic or serve as carcinogens in the human body [11].

As for studies of lipid metabolism related to oak tree wood vinegar, administration of vinegar was effective not only to inhibit triglyceride (TG), total cholesterol (TC), and low density lipoprotein-cholesterol (LDL-C), but also to enhance high density lipoprotein-cholesterol (HDL-C), and therefore effective in the prevention of metabolic syndrome [4]. The principal component of oak tree wood vinegar, except for water, is acetic acid [4], a component that is reported to reduce blood TC and TG [8].

Oak tree wood vinegar is also related to antioxidant activities. A study of the antibacterial activity and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenger activity of the vinegar reported that the antioxidant activities of the vinegar were the highest mainly in phenolic fraction [22]. Another study reported that the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in liver tissues were enhanced [3]. According to a study of the antibacterial and antioxidant activities of oak
tree wood vinegar, measurement of SOD-like activities showed that enhancement of extract concentration increased the antioxidant effects [16]. Meanwhile, Tagliazucchi [35] reported that polyphenol in vinegar inhibited oxidative stress that was produced during the digestion by pepsin. As mentioned above, oak tree wood vinegar begins to be edible due to the scientific approaches, but there are few relevant studies because of its earlier stage. In addition, few scientific approaches have been attempted in spite of interests in health. Especially, there is few research of related to the exercise training and wood vinegar ingestion. Therefore, in this study, we examined the influence of oak tree wood vinegar as a natural food on blood lipids concentration and the effect of inhibition of lipid peroxidation that could be induced by exercise.

Materials and Methods

Animal care

For this study, 28 male rats, aged five-week of Sprague-Dawley (SD) were purchased from Hyochang Science (Korea) as the subjects. The rats of the same age and the weight from 120 to 140 g were purchased, and preliminary breeding had been carried out for two weeks before group classification.

All the rats were individually bred in cages in order to provide the same stress from joint breeding as much as possible. The light and dark cycle of the rats was set (08:00-20:00 for the dark period and 20:00-08:00 for the light period), and the rats were induced to adjust the environment for four weeks by automatic control of lighting under the living circumstances identical to those of human. Cages were changed in their platforms every week to be identical in their lighting environmental stress. The temperature of the breeding room was 23-25°C and the relative humidity was around 60%. The feces were removed every two days for optimum circumstances.

Feeding

Pellet chow (Sam Yang, Republic of Korea) for experimental small animals during the preliminary breeding was used as the feedstuff. During that period, the rats were freely fed of water and feed, and the feed was replenished before the rest was judged to be insufficient. Water was replenished when the bottles were half empty and 200 ml of water was basically provided at a time. In this study, the rats underwent restrict diet by 10 g of feed in the morning and in the afternoon for providing identical calories. The high-fat diet used in this study (C: 43%, F: 40%, P: 17%) was made by the basis of the tables of food composition (AIN-76). The rats were divided into the control group (CON), the exercise training group (EXE), the vinegar ingestion group (VIN), and the vinegar ingestion and exercise training group (VINEXE). The vinegar used in this study was the 100% oak tree wood vinegar made by K Company (Korea), and the test solution was made by mixture of the 100% undiluted solution with distilled water to make 35% solution, which then was diluted to be 50% in concentration.

Oak tree wood vinegar was orally administered by 1 ml one hour before each exercise for four weeks between the beginning and the end of exercise training by using of zonde for oral administration. The control group was orally administered with the same amount of water to maintain identical stress from forced feeding. The meal was removed 12 hours before sacrifice. Table 1 shows the ingredients of the meal.

Exercise training

The rats underwent exercise training on the motor-driven treadmill for small animals (10 lanes with 12 cm in breadth, 10 cm in height, and 1 m in length). The exercise training was performed five times per week, for four weeks. The intensity of exercise, 10 m/min training was provided in the beginning of the experiment at 8% incline and 27 m/min training was provided in the 4th week, under the principle of incremental exercise program.

Sample collection

After decapitation, the blood of the rats—mixture of arterial and venous bloods—were collected. Then, the abdomen was cut open, and part of the liver was extracted, clamped, suspended of its activation in liquid nitrogen, measured in its weight, and preserved in a -70°C freezer (Sanyo, Japan) for the future analysis. The liver weight was measured by using a micro-balance (1/10,000, Shimadzu, Japan). The collected blood was stocked in ice, and after sacrificing each group, seven samples were collected, centrifuged by using a centrifuge (Vision Science, Korea) at 3,000 rpm for 15 min, and supernatant was stored in a freezer with other tissues for the future analysis.

The weight of fats (mesentary, epididymal, and peri-renal) was quantified, and glucose was analyzed by using
Table 1. Diet composition

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein(^1) (g)</td>
<td>204.00</td>
</tr>
<tr>
<td>Corn-starch(^2) (g)</td>
<td>460.00</td>
</tr>
<tr>
<td>Cellulose (g)</td>
<td>30.00</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>50.00</td>
</tr>
<tr>
<td>Corn oil (g)</td>
<td>211.00</td>
</tr>
<tr>
<td>Mineral mix(^3) (g)</td>
<td>35.00</td>
</tr>
<tr>
<td>Vitamin mix(^3) (g)</td>
<td>10.00</td>
</tr>
<tr>
<td>DL-methionine(^3) (g)</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

\(^1\)Sam Yang, Seoul, Korea, \(^2\)Casein high protein, Teklad Test Diet, Madison, Wisconsin, USA
\(^3\)AIN-76 (g/kg mixture): Calcium phosphate, dibasic (CaHPO\(_4\) \cdot 2H\(_2\)O) 500, Sodium chloride (NaCl) 74, Potassium citrate monohydrate (K\(_2\)C\(_6\)H\(_7\)O\(_7\) \cdot H\(_2\)O) 220, Potassium sulfate (K\(_2\)SO\(_4\)) 52, Magnesium oxide (MgO) 24, Maranous carbonate (45-48% Mn) 3.5, Ferric citrate (16-17% Fe) 6, Zinc carbonate (70% ZnO) 1.6, Citric carbonate (53-55% Cu) 0.3, Potassium iodate (KIO\(_3\)) 0.01, Sodium selenite (Na\(_2\)SeO\(_3\) \cdot 5H\(_2\)O) 0.01, Chromium potassium sulfate [Cr\(_2\)(SO\(_4\)) \cdot 12H\(_2\)O] 0.55, filled up to 1,000 with sucrose, Harlan Teklad Co.
\(^4\)AIN-76A (g/kg mixture): β-aminobenzoic Acid 11.0132, ascorbic acid, coated (97.5%) 101.6604, Biotin 0.0441, Vitamin B\(_12\) (0.1% titration in mannitol) 2.9726, Calcium pantothenate 6.6079, Choline dihydrogen citrate 349.6916, Folic acid 0.1982, Inositol 11.0132, Menadione 4.9559, Niacin 9.9119, Pyridoxine HCl 2.2026, Riboflavin 2.2026, Thiamin HCl 2.2026, Dry vitamin A palmitate (500,000 U/g) 3.9648, Dry vitamin D\(_3\) (500,000 U/g) 0.4405, Dry vitamin E acetate (500 U/g) 24.2291, Corn starch, Harlan Teklad Co.
\(^5\)Sigma Chemical Co., St Louis, Missouri, USA

an analysis kit (Sigma, USA). Serum sample, 0.02 ml, was mixed with 3 ml of enzyme reagent, left at 37°C for 5 min, and analyzed for optical density (OD) by using a spectrophotometer (UV-1201, Shimadzu, Japan) at 550 nm in wavelength. Ammonia was analyzed by using an analysis kit (Sigma, USA). 40 ml of deproteinised reagent and 1.0 ml of blood sample were mixed sufficiently, centrifuged by using a centrifuge (Vision Science, Korea) at 2,500 rpm for 5 min, mixed with a color-developing reagent, left at 37°C for 20 min, and measured of OD by using a spectrophotometer at 630 nm in wavelength. TC, TG, and HDL-C were analyzed as blood lipids. An analysis kit (Eiken Co., Japan) was used with given methods. LDL-C was analyzed by using the LDL-C estimating formula presented by Friedewald et al. [6]: LDL-C (mg/dl) = [(TC)-(HDL-C)] - (TG / 5). Free fatty acids (FFA) was also analyzed by using an analysis kit (Shinyang Chimical, Korea).

As a preproces for analysis of lipid peroxidation, a liver tissue sample was quantified of the weight, distilled at 10% by using 0.25 M sucrose, 0.5 mM EDTA, and 5 mM HEPES, centrifuged by using a centrifuge at 8,000× g for 20 min, and collected of 2.0 ml of supernatant for analyzing MDA (malondialdehyde). The rest was centrifuged at 10,000× g for 30 min, and 1 ml of supernatant was mixed with 0.25 ml of ethanol and 0.15 ml of chloroform for two minutes, centrifuged at 10,000× g for 30 min, and 1.0 ml of supernatant was collected for analyzing SOD. All procedures were performed on ice for suspending its activities.

MDA was analyzed by using the method presented by Saitoh [33], 0.5 ml of the sample was mixed with 2.5 ml of 10% TCA solution, reacted at normal temperature for 10 min, and centrifuged at 3,500 rpm for 15 min to be removed of the supernatant. The sediment was mixed with 0.05 M H\(_2\)SO\(_4\) (2.5 ml), and centrifuged at 3,500 rpm for 10 min to be removed of the supernatant. Then, the sediment was mixed with 0.05 M H\(_2\)SO\(_4\) (2.5 ml) and 3 ml of TBA, was heated at 95°C for 30 min, cooled under cold water, mixed with 3 ml of n-butanol : pyridine (15:1 V/V), and centrifuged at 3,000 rpm for 10 min. Then the supernatant was collected to be measured of OD by using a spectrophotometer at 530 nm.

SOD was analyzed by using the method presented by Marklund et al. [26]. Sample, 0.1 ml, was mixed with Tris-EDTA-HCl buffer (pH 8.5), that was mixed with 50 mM Tris (hydroxymethyl)-aminomethan with 10 mM EDTA and 1 N HCl, mixed with 0.1 ml of pyrogallol solution, and it was heated at 25°C for 10 min, mixed with 0.05 ml of 1 N HCl, and then measured of OD at 420 nm. All samples were measured by Lowry et al. [25]. After 20 µl of serum fraction was collected, the serum was mixed with 16 µl of 1.0% SDS and diluted by eight times with 124 µl of distilled water. The serum (15 and 20 µl) was diluted by distilled water (80 and 85 µl), and added by reagent (0.5% copper sulfate solution : 1.0% sodium tartrate solution : 2.0% sodium carbonate solution = 0.5:0.5:49 v/v) by 0.1 ml at a time to be mixed for 10 sec. The solution was left at room temperature for 20 min, mixed with reagent (1.0 N Folin) by 0.1 ml at a time, left at room temperature for 30 min, and measured for OD by using a spectrophotometer at 525 nm for quantification of protein contents by standard calibration curves.

The glycogen in liver was analyzed by the method of Lo et al. [24]. A sample was added by 30% KOH solution, boiled in a water tank at about 100°C for 30 min, mixed sufficiently, mixed with 95% ethanol sufficiently, and wrapped by using paraffin for cold storage for at least six hours. Then, the
sample was centrifuged at 3,000 rpm for about 30 min to be removed of the supernatant and infused of distilled water to be mixed. A certain amount of the mixed sample was collected to be mixed with 5% phenol sufficiently, and added by sulphuric acid to be analyzed at 490 nm. The measured wavelength was substituted to the regression equation that was calculated by tissue amount and standard solutions used for producing glycogen contents.

**Statistical analysis**

All the data were processed by using of the MINITAB (Ver. 13.1, Minitab Inc., USA) for Windows, and the data were represented by mean±standard error of mean (SEM). The one-way ANOVA was used in order to compare all the variables of each group, and tukey test was performed as post-hoc test when there was significant difference. The significance level of all the differences was p<0.05.

**Results**

**Weight changes**

The changes in weight during the experiment are shown in Figure 1. During the 28 days, the VINEXE group showed less weight gain when compared to the CON, the EXE, and the VIN groups, and between the 25th day and the end day it showed statistically significantly low gain when compared to the other groups (p<0.05). However, no significant difference was found among the other groups.

As for the amount of weight gain, the VIN and the VINEXE showed significant inhibition of gain when compared to the CON and the EXE (p<0.05), and that of the VINEXE showed the lowest gain amount (p<0.05). Given that no significant difference was expressed among the whole groups in food efficiency, there might be no effects by meal. The CON group showed significantly higher in mesentary, epididymal, and perirenal fat (p<0.05), and epididymal and perirenal fat was significantly inhibited only by vinegar (p<0.05). In this case the stored fat amount of the VINEXE was significantly lower (p<0.05), and consequently the total fat amount showed the same results.

**Changes in blood profiles**

The CON was significantly high in glucose, which was significantly inhibited by exercise and/or vinegar (p<0.05). Ammonia was slightly high by exercise but significantly inhibited by vinegar (p<0.05). FFA showed high by exercise and/or vinegar (p<0.05), but there was no synergy effect. TG was shown to be significantly inhibited by exercise and/or vinegar, respectively (p<0.05). TC, when compared to the CON group, was shown to be restrained by exercise and/or vinegar (p<0.05), and the VINEXE showed the lowest concentration (p<0.05). HDL-C was shown to be improved by exercise (p<0.05) but no effects by vinegar was expressed. As for LDL-C, when compared to that of the CON, that of the EXE, the VIN, and the VINEXE was significantly restrained (p<0.05), and synergism between the two substances was shown (p<0.05).

Table 2. Differences in body and fat weight

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>EXE</th>
<th>VIN</th>
<th>VINEXE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g)</td>
<td>136.29±5.76*</td>
<td>118.14±4.47*</td>
<td>107.07±2.92*</td>
<td>83.50±7.51*</td>
</tr>
<tr>
<td>FER (%)</td>
<td>0.38±0.02*</td>
<td>0.32±0.02*</td>
<td>0.35±0.03*</td>
<td>0.30±0.04*</td>
</tr>
<tr>
<td>Mesentary fat (g)</td>
<td>5.77±0.39*</td>
<td>5.28±0.29*</td>
<td>5.08±0.14*</td>
<td>3.77±0.17*</td>
</tr>
<tr>
<td>Epididymal fat (g)</td>
<td>3.73±0.22*</td>
<td>2.78±0.15*</td>
<td>2.39±0.16*</td>
<td>1.96±0.12*</td>
</tr>
<tr>
<td>Perirenal fat (g)</td>
<td>4.69±0.37*</td>
<td>3.49±0.22*</td>
<td>2.96±0.13*</td>
<td>1.74±0.10*</td>
</tr>
<tr>
<td>Sum of fats (g)</td>
<td>14.19±0.78*</td>
<td>11.56±0.53*</td>
<td>10.43±0.25*</td>
<td>7.48±0.35*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Group names were described in Fig. 1. FER: food efficiency ratio. Different superscripts has the significance within groups at p<0.05 level, respectively.
Table 3. Changes in blood glucose, ammonia, and lipids within groups

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>EXE</th>
<th>VIN</th>
<th>VINEXE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>116.88±2.77b</td>
<td>101.64±0.58b</td>
<td>92.25±1.78b</td>
<td>86.46±0.96b</td>
</tr>
<tr>
<td>Ammonia (mg/dl)</td>
<td>171.75±4.83b</td>
<td>184.05±6.53b</td>
<td>153.58±3.34b</td>
<td>161.11±5.01c</td>
</tr>
<tr>
<td>FFA (μEq/dl)</td>
<td>597.20±22.70b</td>
<td>748.80±40.20b</td>
<td>858.50±24.70b</td>
<td>942.80±26.70b</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>42.04±1.10b</td>
<td>33.42±1.00b</td>
<td>30.08±1.40b</td>
<td>31.08±1.03b</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>60.41±1.78b</td>
<td>48.94±2.04b</td>
<td>45.01±0.96bc</td>
<td>41.91±1.64b</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>17.05±0.86b</td>
<td>21.37±0.78b</td>
<td>17.94±0.79b</td>
<td>20.92±0.72b</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>34.96±1.59b</td>
<td>20.88±1.66b</td>
<td>21.06±1.26b</td>
<td>14.77±2.02b</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Group names were described in Fig. 1.
FFA: free fatty acids; TG: triglyceride; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.
Different superscripts have the significance within groups at p<0.05 level, respectively.

Table 4. Changes in MDA, SOD, and glycogen contents

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>EXE</th>
<th>VIN</th>
<th>VINEXE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA content (nm/mg protein)</td>
<td>5.41±0.19b</td>
<td>4.62±0.03b</td>
<td>2.95±0.27b</td>
<td>1.74±0.14d</td>
</tr>
<tr>
<td>SOD activity (U/mg protein/min)</td>
<td>17.51±0.09b</td>
<td>20.27±0.37b</td>
<td>23.56±0.81b</td>
<td>26.22±1.23b</td>
</tr>
<tr>
<td>Glycogen content (mg/g)</td>
<td>65.90±3.20b</td>
<td>73.73±2.23b</td>
<td>71.28±1.56b</td>
<td>68.34±3.12d</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Group names were described in Fig. 1.
Different superscripts have the significance within groups at p<0.05 level, respectively.

Changes in liver MDA, SOD, and glycogen content

MDA content was shown to be inhibited by exercise, and significant difference was expressed among each group (p <0.05). SOD activity was shown to be significantly improved by exercise and/or vinegar (p<0.05). The synergy effect between the two substances was observed (p<0.05). Meanwhile, glycogen content showed no significant effects.

Discussion

In the present study, we investigated the effects of exercise training and oak tree wood vinegar ingestion for four weeks on the blood lipids and lipid peroxides. For doing this, 28 SD male rats were selected as the subjects that were orally administered of oak tree wood vinegar with treadmill running training and high fat diet for two weeks, and sacrificed for collecting data.

Cho and Choi [4] reported that the weight gain was reduced according to the amount of administration of oak tree wood vinegar but they did not suggest discussion on its direct causes. Kim [18] reported an experiment of toxicity of refined oak tree wood vinegar in which ICR mice were administered with the vinegar for four weeks with different amounts. In the study, mice that were administered with 1.0 g/kg had been significantly reduced of their weight gain since the 21st day, and 5.0 g/kg showed significant difference on the 28th day. In the present study, no statistically significant difference was shown among the all groups during the whole period, but on the 25th day statistically significant difference was expressed between the CON and the VINEXE, a result that was similar to that of Kim [18]. Despite some extent of difference, Kim et al. [17] reported that when mice were injected with sarcoma-180 tumor cells and administered with oak tree wood vinegar in order to investigate changes in weight and life extension (mean survival days and prolongation of life), the weight gain was significantly reduced when compared to that of the control group, which showed effects of inhibiting proliferation of the tumor cells. Also, the study reported that the mean life span of the control group and the experiment group was 16.13 and 18.25 days, respectively. In particular, although no significant difference was shown among the groups based on the food efficiency, the weight of fat tissue of the VINEXE was significantly reduced when compared to that of the EXE, which indicated that oak tree wood vinegar ingestion with exercise will enhance the stored fat utilization.

Johnston [13] and Johnston et al. [15] reported that when normal people, patients with insulin resistance, and type 2 diabetic patients were administered with the same amount
of apple vinegar in order to investigate their blood glucose and insulin response, glucose and insulin concentrations significantly decreased more than the placebo. According to Ostman et al. [31], vinegar ingestion delayed glucose and insulin responses as postprandial metabolites, and the higher vinegar concentration was, the more significant the differences were. Given that the main ingredient of vinegar was acetic acid [8], such results were reported that acetic acid inhibited disaccharide activation and enhanced the concentration of G-6-P (glucose-6-phosphate) in skeletal muscles. The G-6-P in skeletal muscles is an enzyme to function not only in glycogen breakdown but synthesis [9]. According to Johnston and Buller’s study [14] on effects of vinegar ingestion from another aspect, the subjects who ate bagel and juice after vinegar ingestion showed significant reduction in their glucose concentration when compared to the control group, and the subjects who ate chicken and rice showed insignificant but lower glucose concentration. The whole energy intake was lowered by around 200 kcal, although insignificant, when vinegar was ingested. Such effect is caused by the fact that the acetic acid in vinegar disturbs disaccharide absorption in small intestine [28], or by the fact that glucose inflow stimulus and utilization in peripheral tissues increase [9]. Also in this study, glucose concentration was significantly declined by administration of oak tree wood vinegar, and exercise training with administration of the vinegar showed significant reduction in the concentration. Such results may be caused by the acetic acid of oak tree wood vinegar, as mentioned above. The glucose reduction by acetic acid may be sufficiently effective in prevention and treatment of overweight and obesity. According to Ostman et al. [31], when people ate bread with one or two spoonful of vinegar, the blood glucose concentration was sufficiently low and their postprandial satiety was prolonged by more than twice when compared to those who only ate bread. Low postprandial glucose concentration may not only delay oxidative stress induced by high postprandial glucose concentration but prolong satiety to maintain low calorie intake for about two to four hours [30].

Oak tree wood vinegar was 3 to 4 in pH [11], and the vinegar used in this study was 3 in pH. When the vinegar is 5.0 or less in pH, the generation of ammonia is declined if not in fermentation [37]. Chyla and Vrzgula [2] reported that when 2 ml/kg of acetic acid was administered to sheep, the actual pH was declined and the toxicity by ammonia was considerably reduced because the production of ammoni was inhibited. In our study, the ammonia concentration of the VIN was significantly low when compared to that of the CON, and similarly, its concentration was significantly low in cases of administration of the vinegar accompanied with exercise. Such results are correspondent with those presented in previous studies, suggesting that the vinegar may effectively inhibit fatigue of the central nervous system by exercise.

Since a few years ago, postprandial blood lipid concentration has been warned to be significantly relevant to cardiovascular diseases and the risk of high fat diet has been focused on because such diet increases postprandial blood concentrations of TG and its byproducts [1]. According to Cho and Choi’s study [4] on lipid metabolism of oak tree wood vinegar, TG, TC, and LDL-C were effectively inhibited and HDL-C was effectively enhanced. The results indicate that the vinegar may be effective in prevention of metabolic syndrome. In the present study, TG concentration was significantly declined in the VIN when compared to the CON, indicating the same results of above-mentioned study. Also, TC and LDL-C were shown to decrease by administration of the vinegar. However, there were no significant differences in TG and TC between the VIN and VINEXE, and the single effect of oak tree wood vinegar was more considerable than the effect of exercise. It may be acetic acid that affects the decline in blood lipid concentration by administration of oak tree wood vinegar. The main ingredient of the vinegar is acetic acid when water is excluded [4], which is reported to decline blood TC and TG [8]. According to Fushimi et al. [8], when mice were fed high-cholesterol diet and acetic acid, the TC and TG were significantly declined when compared to those of the control group that were ingested with only cholesterol diet, and the secreted amount of stool to bile acid significantly increased because first, lipogenesis in liver was restrained and second, discharge of cholesterol as stool was enhanced. Cho and Choi [4] also indicated that acetic acid might be a substance among the active ingredients of oak tree wood vinegar in reduction of blood lipid concentration. According to Kim et al. [20], on acidic fraction of oak tree wood vinegar, propionic acid, acetic acid, and n-butyric acid occupy around half of the whole contents. Jodai et al. [12] reported that acetic acid occupied 51% the maximum, indicating that the content of acetic acid in oak tree wood vinegar was considerably high. Acetic acid is a short-chain fatty acid and is produced by fermentation in stool or after inflow of dietary fibers in the large intestine.
It is reported that plenty of fermentable fibers restrain increase in blood cholesterol [8]. Therefore, its effectiveness of decline in blood lipids, acetic acid of oak tree wood vinegar in this study may play an important role in reduction in blood lipids. Or, when considering the effects of phenolic compounds, the main components of the vinegar are 35.4% in acidic fraction, 41.3% in phenol fraction, 19.5% in neutral fraction, and 3.8% in basic fraction from Japanese cedar, while 25.3%, 47.0%, 24.8%, and 2.9%, respectively, from oak tree [20]. Given that plenty of phenol compounds are contained in the vinegar (another characteristic of the vinegar), Shen et al. [34] reported that tomato contained plenty of phenolic compounds and that after tomato was administered for six weeks, no significant change was shown in TC concentration but TG and LDL-C were significantly declined while HDL-C was significantly enhanced. When considering a report in which administration of cinnamate, a phenolic compound contained in cinnamon increased HDL-C and consequently declined total cholesterol [21], the decline of TC in our study might be also affected by the increase in HDL-C of the VINEXE group.

Meanwhile, Fushimi and Sato [7] reported that acetic acid declined increase in malonyl-CoA in liver in the postprandial state. According to Fushimi et al. [8], acetic acid significantly declined fatty acid synthase mRNA and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) as a matrix of cholesterol composition was significantly reduced, a result that might demonstrate the possibility. In addition, the results that acyl-CoA oxidase mRNA was significantly enhanced by acetic acid might indicate that dietary acetic acid weakened TG production in the liver that was mediated by cholesterol and that consequently blood TG concentration was declined [8].

The considerably high concentration of blood lipids in the postprandial state enhances oxidative stress and in consequence risk in cardiovascular diseases [1]. Various diseases including brain and cardiac diseases such as cancer, diabetes, cerebral apoplexy, myocardial infarction, hepatitis, nephritis, and Parkinson’s disease, ischemia, arteriosclerosis, skin diseases, digestive diseases, and inflammation are reported to be relevant to oxygen free radical. Recently, studies have been performed on antioxidant activation of oak tree wood vinegar, and Jeong and Shim [11] reported that antioxidant effects were higher in the groups of addition of 1% and 5% of the vinegar when compared to the control group and that such results might indicate that the vinegar removed oxygen free radical within human body. According to Cho and Choi [3], on the effects of pyrolytic liquor on oxygen radicals and their scavenger enzymes in the liver of CD rats, the SOD, GPx, and CAT activations in the liver of the groups of the liquor administration by 25 and 50% in concentration were significantly enhanced when compared to those of the control group, a result that indicated inhibition of oxygen free radical, and that the generations of superoxide radical, hydroxyl radical, and H2O2 were significantly inhibited in liver. Also, Oh and Chung [29] reported that administration of oak tree wood vinegar increased the reduction of oxygen free radical while the vinegar showed around 53% in effect at 700 ppm. According to Jung et al. [16], when the concentration of the extract from oak tree wood vinegar was higher, the antioxidant activities were more enhanced while the vinegar showed around 65% in antioxidant effects. Most vinegar is expressed to prevent oxidative damage in erythrocyte, to promote proteolysis by pepsin, and to reduce hydroperoxide generation due to polyphenol contained in vinegar [35]. He reported that polyphenol in vinegar inhibited oxidative stress that was produced during the digestion by pepsin and enhanced proteino lysis. In the present study, MDA was not only reduced by exercise but significantly reduced by administration of oak tree wood vinegar, while SOD was not only enhanced by exercise but significantly enhanced by administration of the vinegar. Such results may be induced by administration of oak tree wood vinegar affecting on decline in blood lipid concentration and consequent inhibition of oxidative stress [1].

Meanwhile, according to Kim et al. [20], on the phenol fractions of the vinegars from Japanese cedar and oak tree, a total 32 phenol compounds were investigated and the content of phenol was 28.52% in the Japanese cedar vinegar and 23.87% in the oak tree wood vinegar. A research reported that six-week ingestion of tomato containing plenty of phenol compounds such as oak tree wood vinegar significantly improved antioxidation [34]. According to Lee et al. [22], antioxidant activities of oak tree wood vinegar were significantly improved because the phenol fraction of the vinegar contained plenty of substances with antioxidation. Therefore, effects of phenolic compounds may not be excluded in inhibition of lipid peroxidation by administration of oak tree wood vinegar.

Glycogen serves as main energy source in skeletal muscles, and thus exhaustion of glycogen in liver and muscles
leads to fatigue in central nerves and peripheral tissues. Ingestion of vinegar or acetic acid is reported to be sufficiently effective on glycogen restoration [9]. They reported that acetic acid administration significantly enhanced the glycogen contents in liver, gastrocnemius muscles, and soleus muscles, and in particular, acetic acid administration of 0.2 g/100 g diet significantly increased glycogen contents in liver and gastrocnemius muscles even when compared to those in the postprandial state. Orally administered acetic acid is rapidly absorbed, used in liver and peripheral tissues [32], and then metabolized by acetyl-CoA in the TCA cycle in liver and muscles [5]. According to in vitro experiments, acetic acid inhibits activation of phosphofructokinase (PFK) [36], and in that process it is regarded that the amount of glycogen synthesis is enhanced. Fushimi et al. [9] reported that oak tree wood vinegar reduced the concentration of fructose 2,6-bisphosphate in liver, inhibiting glycogen degradation in liver. On the other hand, Fushimi and Sato [7] reported that acetic acid declined the increase in malonyl-CoA in liver in the postprandial state, a result indicating that carbohydrate oxidation was delayed by acetic acid. Malonyl-CoA serves as an inhibitor of carnitine palmitoyl transferase I (CPT-I) that contributes to the oxidation of long-chain fatty acids [27], and inhibition of increase in malonyl-CoA may indicate maintaining the activation of CPT-I and consequent continuation of long-chain fatty acid oxidation. According to Fushimi et al. [10], when glucose or mixture of glucose and acetic acid were administered to mice after seven-day swimming exercise, the glycogen restoration of soleus muscle of mice that were administered with the mixture showed significance increase, with significant enhancement in glycogen synthetase. However in our study, no significant increase was shown, because increase in energy efficiency by fat rather than energy from carbohydrate was centered on due to the high fat diet selected in this study.

As a result, long-term ingestion of the vinegar may reduce blood lipids and lower blood glucose concentration to prevent various diseases, and such effects are suggested to be enhanced when exercise is accompanied. Meanwhile, inhibition of lipid peroxidation by ingestion of oak tree wood vinegar may indicate direct relevance to longevity. Given that few researches have been reported on oak tree wood vinegar, the influence of vinegar may be enhanced through various approaches to the vinegar as a natural food.

References


초록: 4주간 현충의 목초액 섭취와 운동에 따른 혈중지질과 MDA, SOD 활성 차이

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본 연구는 운동훈련과 목초액의 정기섭취가 혈중 지질성분 및 항산화활성에 미치는 영향을 살펴보고자 하였다. 28마리의 Sprague Dawley 수컷 쥐를 이용하여 대조군(CON), 운동군(EXE), 목초액섭취군(VIN), 그리고 목초액과 운동군을 병행한 군(VINEXE)으로 각각 7마리씩 구분하였다. 식이는 고지방식이(40%)를 기본으로 하였으며, 운동군은 4주간의 트레드밀 달리기하였으며, 목초액은 경구투여하였다. 목초액을 섭취하지 않는 집단에게는 동일한 양의 종류수를 경구투여하여 동일한 스트레스를 유지할 수 있도록 하였다. 체중의 변화는 운동 후반기인 CON군에 비하여 VINEXE이 유의하게 적게되는 것으로 나타났다(p<0.05). 지방량의 변화는 VIN 및 EXE군에서 유의한 상승의 억제가 나타났으며(p<0.05), VINEXE 역시 동일한 결과가 나타났다(p<0.05). 혈액성분 중 글루코스와 알코스는 CON
에 비하여 다른 집단에서 유의하게 낮은 것으로 나타났으며(p<0.05). 혈중 지질 중 TC, LDL-C는 CON군에 비하여 다른 집단이 유의하게 낮은 것으로 나타났으며, VINEXE의 유의한 상승효과가 나타났다(p<0.05). HDL-C는 EXE군에서 유의하게 개선되는 것으로 나타났다(p<0.05). MDA 환황은 각각의 집단간 모두 유의한 변화를 보였으며(p<0.05), SOD 활성은 VIN과 VINEXE가 다른 집단에 비하여 유의하게 높은 것으로 나타났다(p<0.05). 이상과 같은 결과를 볼 때 4주간의 운동을 하는 동안 목초액 섭취는 체중 증가 억제 및 혈중 지질성분의 개선, 그리고 지질과산화 억제로 나타나며, 건강 증진에 도움을 줄 수 있을 것이라는 점을 시사하고 있다.