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Improvement of Insulin Resistance and Insulin Secretion by Water Extracts of *Cordyceps militaris*, *Phellinus linteus*, and *Paecilomyces tenuipes* in 90% Pancreatectomized Rats

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The effect of supplementation with *Phellinus linteus* (*P. linteus*), *Paecilomyces tenuipes* (*P. tenuipes*), and *Cordyceps militaris* (*C. militaris*) mushroom water extracts on the insulin secretion and insulin resistance of 90% pancreatectomized (Px) male Sprague Dawley rats was investigated. Px rats were daily administered 0.5 g of *P. linteus*, *P. tenuipes*, and *C. militaris* aqueous extracts or a placebo per 1 kg body weight with a 40% fat diet for 8 weeks. Fasting serum glucose levels were lower in rats receiving *C. militaris* than in the control group. Insulin secretion at the elevated serum glucose levels was lowest in rats that consumed *P. tenuipes* in hyperglycemic clamp. Whole body glucose disposal rates increased in *C. militaris* but decreased in *P. tenuipes* compared to those in the control group in euglycemic hyperinsulinemic clamp. The GLUT4 content and fraction velocity of glycogen synthase in the soleus and quadriceps muscles increased in the rats treated with *C. militaris*, but *P. tenuipes* decreased both. In sum, a water extract of *C. militaris* ameliorates insulin resistance by enhancing glucose utilization in skeletal muscles.

Key words: euglycemic hyperinsulinemic clamp; hyperglycemic clamp; glycogen synthase; GLUT4; mushroom

The pathophysiology of type 2 diabetes mellitus is complicated. There exist few type 2 diabetic patients with pancreatic β-cell defects or insulin resistance exclusively, while in fact these two factors are interlinked. In healthy people, insulin secretion from pancreatic β-cells increases in order to maintain normoglycemia when insulin resistance increases. But the development of type 2 diabetes mellitus results from increased blood glucose levels when insulin secretion cannot compensate for insulin resistance.[1–3] In order to use plants or herbs as treatment for type 2 diabetes mellitus, insulin resistance must be alleviated and/or insulin release enhanced. This leads to a decrease in fasting and post-prandial serum glucose levels.

Herbs have commonly been used as therapeutic agents for various diseases in Asia. Herb treatments have become popular in western countries as well. Recently published research reported that 31% of diabetes patients attending a diabetes education program over a six-month period at the University of Alberta in Canada were taking alternative medications. The patients considered alternative medications efficacious, and they spent almost as much money on over-the-counter supplements and alternative medications together as they did on their diabetic medications.[4] It is important to evaluate these remedies to establish what merit they have and to determine the mechanisms of their action.

Mushrooms have been used widely as nutritional supplements that are claimed to be beneficial for preventing and/or delaying cancer and metabolic diseases such as diabetes and cardiovascular diseases. We studied three mushrooms, *Phellinus linteus* (*P. linteus*), *Paecilomyces tenuipes* (*P. tenuipes*), and *Cordyceps militaris* (*C. militaris*) for anti-diabetic effects, since they have been gaining popularity recently for presumably curing many diseases, without scientific evidence. *C. militaris* and *P. tenuipes* are caterpillar fungi which derive nutrients from host bodies while growing in the host insects. *P. linteus* is included in the Phellinus genus of the Hymenochaetaceae family of the Aphylloporales order in the mushroom class (Basidiomycetes), and grows naturally on the trunks of the mulberry. Several studies have shown that *P. linteus* has potent anti-tumor activity[5] and that *C. militaris* has an anti-hyperlipidemia effect.[6] Not many studies have been performed to understand the mechanisms by which water extracts of mushrooms ameliorate hyperglycemia, if they do. The purpose of this study was to determine the effects of *P. linteus*, *P. tenuipes*, and *C. militaris* water extracts on insulin secretion and insulin resistance in 90% pancreatectomized (Px) male Sprague Dawley rats consuming a high fat diet.

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Materials and Methods

Plant materials. Dried fruiting bodies of *P. linteus*, *P. tenuipes*, and *C. militaris* mushrooms were provided by Dr. Jae Mo Sung (Research Institute of Agricultural Science, Kangwon National University, ChoonChun-Si, Korea). The mushrooms were cultivated in specific media after inoculation with 4% (v/v) of the seed culture of *P. linteus*, *P. tenuipes*, or *C. militaris*. *P. linteus* was cultured in media (pH 5.5) containing 4% glucose, 0.3% polypepton, 0.3% yeast extract, 0.05% KH_2PO_4, and 0.05% Na_2HPO_4 at 25 °C for 45 d. *P. tenuipes* and *P. linteus* were cultured in media (pH 5) containing 10 g/l glucose, 5 g/l polypepton, 3 g/l malt extract, and 3 g/l yeast extract at 25 °C on a rotary shaker incubator for 7 d.[7] Dried fruiting bodies of each mushroom was homogenized to a fine powder and 50 g of each was macerated with 1 liter of water at 80 °C for 10 h. The extract was lysophilyzed and stored at −20 °C.

Animals. Seventy male Sprague Dawley rats, weighing 430 ± 56 g, were purchased from the Korean Animal Resources Center, Seoul, Korea, and housed individually in stainless steel cages in a controlled (23 °C; 12 h light and dark cycle) environment. The rats were allowed to acclimate for one week before the commencement of the study. All surgical and experimental procedures were approved by the Animal Care and Use Committee at Hoseo University, Korea.

The rats were anesthetized with ketamine and xylazine (100 and 5 mg/kg body weight), and 90% of their pancreas was removed using the technique of Hosokawa to generate Px rats.[8] The entire splenic and stomach portion of the pancreas was removed with sterilzed cotton swaps. The pancreatic tissue within 2 mm of the common bile duct and extending from the duct to the first part of the duodenum remained. The residual pancreas was anatomically well defined. For the control group, 12 male SD rats received a sham operation that consisted of disengaging the pancreas from the mesentery and gently rubbing it between the fingers. The sham-operated (Sham) rats did not have any symptoms of diabetes. Following the pancreatectomy or sham operation the animals were allowed free access to standard laboratory chow (Sam Yang Co., Kangwon-Do, Korea) and tap water for two weeks. At the end of the two-week recovery period, post-surgery, those with a blood glucose level of less than 9.4 mmol/l were culled from among the Px rats. The Px rats did not have any symptoms of inflammation at post-surgery.

Experimental protocol and diet. Each of the remaining 50 Px rats was randomly assigned to 4 different groups by picking numbers; three groups were supplemented with water extracts of *P. linteus* (n = 13), *P. tenuipes* (n = 12), or *C. militaris* (n = 13) respectively, and the rest group (n = 12) was supplemented with cellulose as a placebo (the control-Px group). Lysophilyzed *P. linteus*, *P. tenuipes*, and *C. militaris* and the placebo were incorporated into the same high fat diet at a concentration of 10 g/kg of diet. Each rat in the *P. linteus*, *P. tenuipes*, and *C. militaris* groups daily consumed approximately 0.5 g per 1 kg of body weight of respective mushroom water extract for 8 weeks. Twelve Sham rats were supplemented with the placebo as a control-Sham group, used to determine whether mushroom water extract supplementation could overcome impaired glucose homeostasis in Px rats and reach the levels of Sham rats.

All rats had free access to a 40% fat diet. A semi-purified diet based on AIN-93 formulation was modified to contain 40 energy% fat.[9] The sources of carbohydrates, protein, and fats were corn starch (DooSan Corn Products Korea, Seoul, Korea), milk casein (Ducksan Pure Chemical Co., Ltd., Ansan-Si, Korea), and shortening made from animal fat (Wellga Inc., SungNam-Si, Korea), respectively. Overnight fasting blood was collected from the cut-tail tips of conscious individual rats weekly at an assigned time to measure serum glucose levels.

Hyperglycemic (HG) clamp. Indwelling catheters were inserted into the jugular vein and carotid artery during the seventh week of mushroom extract or placebo intake respectively.[10] After 6 d of insertion, HG clamp was performed on the rats in an awake, unstressed, and fasting state to evaluate insulin secretion capacity.[11] Continuous infusion of 25% glucose solution was administered into the jugular vein to raise the serum glucose concentration by about 5.6 mmol/l above baseline concentration. Serum samples for determination of glucose were obtained from the carotid artery at 5-min intervals. The serum glucose concentration was subsequently held constant at this hyperglycemic plateau from time 60 to 90 minutes by the adjustment of a variable glucose infusion. Serum insulin concentrations were determined at time 0, 60, and 90 minutes. Serum insulin levels at 60 and 90 minutes represent the insulin secretion capacity of rats when serum glucose levels increased by 5.6 mmol/l. After finishing, HG clamp rats were provided food and the respective fluids for 6 h, and subsequently deprived of food for 16 h.

Euglycemic hyperinsulinemic (EH) clamp. On the day following HG clamp, EH clamp studies were performed to measure whole-body glucose disposal rates in the rats in an awake, unstressed, fasting state. Hyperinsulinemia was achieved with a constant infusion of human insulin (12 mU/kg body weight/min), and euglycemia (5.6 mmol/l) was maintained at a variable rate of 25% glucose solution infusion, adjusting every 5 min according to the serum glucose levels collected from the jugular vein.[12] The total glucose infused equals the total glucose disposal in the tissues of the whole body. The glucose infusion rate was calculated and expressed in

terms of mg of glucose per kg of body weight per min. The glucose disposable rate is an index of the whole-body glucose utilization response to exogenous insulin.

After the EH clamp study, the rats were immediately anesthetized with xylazine (10 mg/kg body weight) and killed by decapitation. Tissues were rapidly removed and frozen in liquid nitrogen, and stored at $-70\,^{\circ}C$ until further analysis was performed.

Biochemical measurements. Serum glucose levels were analyzed with a glucose analyzer (Beckman, Fullerton, CA), and serum insulin levels were measured with commercial radioimmunoassay kits (Linco Research, St. Charles, MO). In order to determine the glycogen levels in the liver and soleus and quadriceps muscle tissues, these tissues were homogenized and deproteinized with 1.5 N perchloric acid. Glycogen was digested into glucose with α-amyloglucosidase in the acid buffer. The glycogen levels were expressed as glucose levels derived from glycogen in the soleus and quadriceps muscle and liver tissues.[13] After removing interstitial fat from the muscle, the intracellular contents of triacyglycerol in the soleus and quadriceps muscles were extracted with a chloroform–methanol solution (2:1, vol/vol), and the extracted triacyglycerol was resuspended in chroloform.[14] Triacyglycerol concentration was determined using a Trinder kit (Sigma, St. Louis, MO). Glycogen synthase activity was measured by a modification of the method of Thomas *et al.*[15] and Frontoni *et al.*[13] After centrifugation of muscle homogenates, the supernatant was incubated with a physiologic concentration of the substrate (0.3 mM UDPG-[^{3}H] glucose) in the presence or absence of 10.0 mM glucose 6-phosphate (G-6-P). Total glycogen synthase activity is calculated by the sum of the G-6-P activities in the presence and absence of 10.0 mM G-6-P, and the activity is expressed as nanomoles per mg protein per min. Fractional velocity (FV) of glycogen synthase is determined as a percentage of the ratio of the activity in the absence of G-6-P and in the presence of 10 mM G-6-P. Total membranes from the soleus and quadriceps muscles were prepared by the method of Walker *et al.*[16] The glucose transporter 4 (GLUT4) content in total membranes was measured by Western blotting with rabbit GLUT4 antibody (Chemicon, Temecula, CA). The second antibody was anti-rabbit immunoglobulin G conjugated with horseradish peroxidase. Immune complexes were detected using an enhanced chemiluminesence detection system followed immediately by several sequential exposures to X-ray film (Eastman-Kodak, Rochester, NY). The muscle standard (an unrelated crude membrane fraction) was run on every gel for comparison of samples from different immunoblots. Quantification was performed with a scanning laser densitometer (BioRad, Richmond, CA).

Statistical analysis. All results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using the SAS statistical analysis program.[17] The mushroom effect was determined by Tukey's test after one-way analysis of variance (ANOVA) had established significant differences among the groups of Px rats. Comparison between Px and Sham rats was performed by a two-sample Student's t-test, where necessary. Differences with a $P < 0.05$ were considered statistically significant.

Results

Mushroom, food, and fluid intakes, and body weight

Diabetic status and mushroom extract supplementation did not affect food intake or daily total caloric intake during the entire mushroom extract administration period (Table 1). Average daily caloric intake was 29.9 ± 2.9 kJ in all rats. Regardless of mushroom water extract supplementation, fluid intake was not different among the Px rats, but was higher in Px rats than in Sham rats. The amount of mushroom extract intake did not differ among the groups supplemented with *P. linteus*, *P. tenuipes*, *C. militaris*, or cellulose in the Px rats. Before and after an eight-week mushroom extract supplementation, the mean body weight of the Px rats was not different among the various groups. Mean body weight did not change in the Px rats during an eight-week mushroom extract supplementation, but it increased in the Sham rats. Body weight was lower in Px rats than in Sham rats at the end of the experimental period (Table 1), possibly due to urinary glucose loss in the post-prandial state of the Px rats.

Serum glucose levels and insulin secretion

Prior to supplementation with mushroom water extracts, fasting serum glucose levels were not different among the groups of Px rats, but were higher in Px rats as than in Sham rats. *C. militaris* lowered serum glucose levels compared to the control-Px group from the fourth week of supplementation, while *P. tenuipes* increased the levels (Fig. 1). Fasting insulin levels were not different among the groups of Px rats before supplementation, but they were significantly lowerer in Px rats than in Sham rats. At the end of the supplementation period, fasting serum insulin levels did not differ among the mushroom extract and placebo groups of Px rats (Table 1).

At the end of the supplementation period, insulin secretion at the steady state of higher serum glucose levels by 5.6 mmol/l above baseline values increased compared to baseline at HG clamp in all groups (Table 2). Serum insulin levels of the steady state were lowest in rats that consumed *P. tenuipes* among all groups administered mushroom water extracts or placebo. The consumption of *P. tenuipes* water extract decreased the insulin secretion capacity of β-cells, which may develop diabetes in insulin resistant states. The capacity was suppressed in Px rats more than in Sham rats.

Table 1. Initial and Final Body Weight, Average Intakes of Fluid and Mushroom Extracts, and Serum Insulin Levels during Mushroom Extract and Placebo Supplementation

	P. linteus	*P. tenuipes*	*C. militaris*	Control-Px	Control-Sham
N	13	12	13	12	12
Body weight, *g*					
Initial	431.2 ± 48.9[1]	434.0 ± 42.8	433.4 ± 40.6	430.4 ± 46.6	454.4 ± 34.3
Final	424.5 ± 29.8	427.1 ± 71.8	412.0 ± 35.8	441.7 ± 46.0	525.6 ± 45.7**
Fluid intake, *ml/d*					
Initial	38.7 ± 1.4	37.6 ± 2.4	39.5 ± 4.3	36.5 ± 3.2	29.5 ± 2.3**
Final	47.3 ± 3.7	49.5 ± 3.5	44.1 ± 3.1	48.4 ± 3.9	33.4 ± 3.3**
Mushroom intake, *mg/d*					
Initial	226 ± 19	235 ± 21	221 ± 17	0	0
Final	254 ± 27	263 ± 23	255 ± 22	0	0
Food intake, *g/d*					
Initial	22.6 ± 1.9	23.5 ± 2.1	22.1 ± 1.7	22.9 ± 2.5	22.5 ± 1.9
Final	25.4 ± 2.7	26.3 ± 2.3	25.5 ± 2.2	26.1 ± 2.9	26.4 ± 2.3
Serum insulin, *pmol/l*					
Initial	275.5 ± 25.2	266.6 ± 22.8	271.2 ± 27.8	268.3 ± 23.1	322.7 ± 36.7*
Final	285.6 ± 30.4	268.8 ± 28.8	255.1 ± 39.1	273.4 ± 30.2	338.1 ± 38.6*

[1]Values are means ± SD.

P. linteus, *Phellinus linteus*; *P. tenuipes*, *Paecilomyces tenuipes*; *C. militaris*, *Cordyceps militaris*; Px, pancreatectomy; Sham, sham operation.

*Significantly different from the control-Px group, $P < 0.01$; **$P < 0.001$ (by *t*-test).

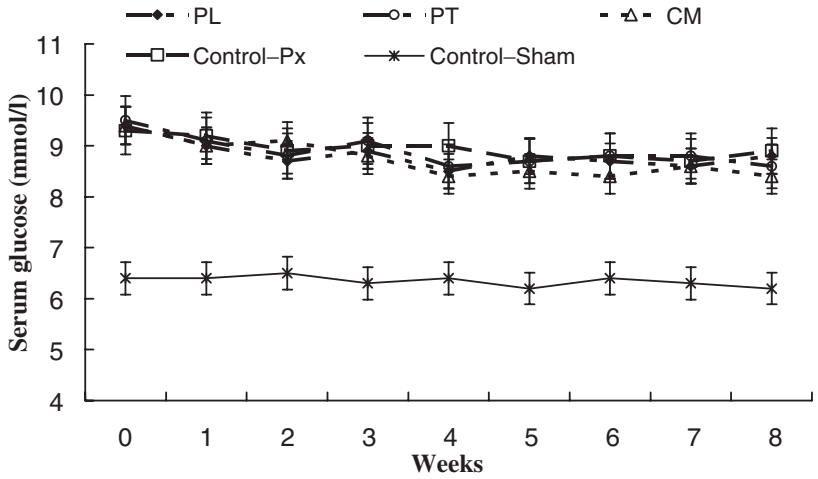

Fig. 1. Changes in Blood Glucose Levels in Five Different Groups during Mushroom Extracts and Placebo Supplementation.

P. linteus, *Phellinus linteus*; *P. tenuipes*, *Paecilomyces tenuipes*; *C. militaris*, *Cordyceps militaris*; Px, pancreatectomy; Sham, sham operation. The control-Sham group ($n = 12$) was significantly different from the control-Px group ($n = 12$), $P < 0.001$. The values for the group supplemented with *C. militaris* group ($n = 13$) were significantly different from those of the groups receiving either *P. linteus* ($n = 13$) or *P. tenuipes* ($n = 12$), $P < 0.05$.

Insulin resistance

Table 3 shows whole body glucose disposal rates at EH clamp, which indicate the degree of insulin resistance. Among the Px rats, glucose disposal rates increased in the order of the *P. tenuipes*, placebo, *P. linteus*, and *C. militaris* supplemented groups. This suggested that *C. militaris* water extract consumption decreased insulin resistance, while *P. tenuipes* increased it more than the placebo in the Px rats. The rates of rats receiving *C. militaris* increased to reach those of the control-Sham group.

At post EH clamp, liver and muscle glycogen levels are shown in Table 4. Soleus and quadriceps muscles were selected for measuring glucose utilization since the soleus and quadriceps muscles represent slow-twitching and fast-twitching muscles respectively.[18] They mainly utilize different energy sources: soleus muscle uses fat as an energy source and quadriceps muscle uses glucose. Liver and quadriceps muscle glycogen levels were not affected by supplementation with mushroom water extracts among the Px rats. Post clamp soleus muscle glycogen levels were significantly higher in the group treated with *C. militaris* than in the control-Px group, while the deposits of the group supplemented with

Table 2. Serum Glucose and Insulin Levels at Hyperglycemic Clamp at the End of the Mushroom Extract and Placebo Supplementation

	P. linteus	*P. tenuipes*	*C. militaris*	Control-Px	Control-Sham
N	13	12	13	12	12
Serum glucose, *mmol/l*					
Baseline	8.4 ± 1.1[1,a,b]	9.4 ± 0.8[a]	8.1 ± 0.9[b]	8.6 ± 1.1[a,b]	6.2 ± 0.9*
Steady-state[2]	13.7 ± 2.1	14.8 ± 2.5	13.8 ± 2.7	14.2 ± 1.2	11.6 ± 1.7
Serum insulin, *pmol/L*					
Baseline	283.6 ± 22.6	269.2 ± 29.2	256.2 ± 30.8	274.8 ± 31.8	325.4 ± 39.3*
Steady-state[2]	356.8 ± 38.8[a]	302.6 ± 36.6[b]	379.2 ± 41.2[a]	342.8 ± 43.5[a]	654.3 ± 45.7**

[1]Values are means ± SD.

P. linteus, *Phellinus linteus*; *P. tenuipes*, *Paecilomyces tenuipes*; *C. militaris*, *Cordyceps militaris*; Px, pancreatectomy; Sham, sham operation.

[a,b]Different superscript letters are significantly different among Px groups, $P < 0.05$ (by Tukey's test).

*Significantly different from the control-Px group, $P < 0.01$; **$P < 0.001$ (by *t*-test).

[2]Steady-state: At the plateau of the serum glucose concentration by about 5.6 mmol/l above baseline fasting glucose concentration with continuous injection of 25% glucose solution.

Table 3. Glucose Disposal Rates and Serum Glucose and Insulin Levels at Euglycemic Hyperinsulinemic Clamp at the End of Mushroom Extract and Placebo Supplementation

	P. linteus	*P. tenuipes*	*C. militaris*	Control-Px	Control-Sham
N	13	12	13	12	12
Glucose disposal rates, *mg/kg BW/min*	14.5 ± 2.7[1,b]	11.2 ± 2.1[c]	19.4 ± 3.4[a]	14.0 ± 2.3[b]	24.2 ± 3.6**
Serum glucose, *mmol/l*					
Baseline	8.7 ± 0.8[a,b]	9.0 ± 0.9[a]	8.2 ± 0.7[b]	8.5 ± 0.8[a,b]	6.4 ± 0.8**
Steady-state[2]	5.9 ± 0.5	5.7 ± 0.8	5.7 ± 1.0	5.7 ± 0.6	5.7 ± 0.5
Serum insulin, *pmol/l*					
Baseline	283.6 ± 31.5	274.7 ± 29.6	262.3 ± 30.8	273.4 ± 28.1	331.1 ± 37.7*
Steady-state[2]	1944 ± 288	1840 ± 274	1886 ± 251	1857 ± 293	2011 ± 263

[1]Values are means ± SD.

P. linteus, *Phellinus linteus*; *P. tenuipes*, *Paecilomyces tenuipes*; *C. militaris*, *Cordyceps militaris*; Px, pancreatectomy; Sham, sham operation.

[a,b,c]Different superscript letters are significantly different among Px groups, $P < 0.05$ (by Tukey's test).

*Significantly different from the control-Px group, $P < 0.01$; **$P < 0.001$ (by *t*-test).

[2]Steady-state: the state to maintain serum glucose levels at 5.6 mmol/l with continuous injection of 12 mU human regular insulin/kg body weight/min.

Table 4. Soleus and Quadriceps Muscle Glycogen, Triacylglycerol, and GLUT4 at the End of Mushroom Extract and Placebo Supplementation

	P. linteus	*P. tenuipes*	*C. militaris*	Control-Px	Control-Sham
N	13	12	13	12	12
Glycogen, *mg/g tissue*					
Liver	38.4 ± 9.8[1]	36.5 ± 7.5	43.5 ± 8.3	40.8 ± 10.7	51.0 ± 11.2*
Soleus M	4.0 ± 0.7[a,b]	3.4 ± 0.8[b]	4.5 ± 0.7[a]	3.8 ± 0.5[b]	5.1 ± 0.9**
Quadriceps M	2.7 ± 0.9	2.6 ± 0.7	3.2 ± 0.9	2.6 ± 0.8	4.9 ± 1.0**
Triglycerides, *mg/g tissue*					
Soleus M	8.6 ± 1.1[a]	9.1 ± 0.9[a]	7.1 ± 1.2[b]	8.5 ± 1.5[a]	7.3 ± 1.3*
Quadriceps M	10.9 ± 1.3	11.7 ± 1.4	10.3 ± 0.9	11.0 ± 1.3	11.4 ± 1.6
GLUT4, %					
Soleus M	92.3 ± 18.6[b]	75.1 ± 14.4[c]	114.8 ± 19.4[a]	91.3 ± 15.7[b]	129.5 ± 18.9**
Quadriceps M	99.7 ± 14.5[b]	85.4 ± 11.7[c]	100.8 ± 15.7[a]	98.6 ± 11.5[b]	121.4 ± 17.3*

[1]Values are means ± SD.

P. linteus, *Phellinus linteus*; *P. tenuipes*, *Paecilomyces tenuipes*; *C. militaris*, *Cordyceps militaris*; Px, pancreatectomy; Sham, sham operation; M, muscle; GLUT4, glucose transporter 4.

[a,b,c]Different superscript letters are significantly different among Px groups, $P < 0.05$ (by Tukey's test).

*Significantly different from the control-Px group, $P < 0.05$; **$P < 0.01$ (by *t*-test).

Table 5. Glycogen Synthase Activity in Soleus and Quadriceps Muscles at the End of Mushroom Extract and Placebo Supplementation

	P. linteus	*P. tenuipes*	*C. militaris*	Control-Px	Control-Sham
N	13	12	13	12	12
Soleus M					
Total activity, *nmol UDPG-G/min/mg tissue*	32.8 ± 5.4[1]	33.2 ± 4.3	35.1 ± 4.9	35.4 ± 6.5	41.8 ± 5.8*
Fraction velocity[2], %	$9.4 \pm 1.4^{b,4}$	8.2 ± 1.2^{c}	11.8 ± 1.7^{a}	$8.7 \pm 1.5^{b,c}$	12.7 ± 1.2**
Quadriceps M					
Total activity, *nmol UDPG-G/min/mg tissue*	31.4 ± 6.6	30.5 ± 5.8	31.4 ± 7.1	32.5 ± 5.9	38.9 ± 6.5*
Fraction velocity[2], %	8.6 ± 1.2^{b}	8.1 ± 1.4^{b}	9.8 ± 1.4^{a}	8.2 ± 1.3^{b}	10.2 ± 1.5*

[1]Values are means ± SD.

P. linteus, *Phellinus linteus*; *P. tenuipes*, *Paecilomyces tenuipes*; *C. militaris*, *Cordyceps militaris*; Px, pancreatectomy; Sham, sham-operation; M, muscle.

[a,b,c]Different superscript letters are significantly different among Px groups, $P < 0.05$ (by Tukey's test).

*Significantly different from the control-Px group, $P < 0.05$; **$P < 0.01$ (by *t*-test).

[2]Percentage of the ratio of the activity in the absence of glucose-6-phosphate (G-6-P) and in the presence of 10 mM G-6-P.

P. tenuipes did not differ from those of the control-Px group. Soleus muscle triacylglycerol levels decreased in Px rats that consumed *C. militaris* water extract more than those consumed placebo, and the levels of the group treated with *C. militaris* were not different from those of the control-Sham group. But quadriceps triacylglycerol levels did not differ among the groups. Decreased triacylglycerol levels in soleus muscles in *C. militaris* treated Px rats indicated that muscle insulin resistance recovered in those rats. Decreased storage of triglycerides in soleus muscle may enhance insulin action better than in quadriceps muscle since soleus muscle utilizes predominantly fats for energy.[19)]

The GLUT4 contents in the soleus and quadriceps muscles of the group treated with *C. militaris* were higher than those of the control-Px group, and their contents increased up to the same levels as the control-Sham. But the GLUT4 contents were lower in the group supplemented with *P. tenuipes* than in the control-Px group in soleus and quadriceps muscle.

Table 5 shows total glycogen synthase activity and fraction velocity of glycogen synthase in soleus and quadriceps muscle tissues after EH clamp. Total glycogen synthase activity decreased in the soleus and quadriceps muscles of the control-Px rats compared to those of the control-Sham rats, but there was no difference in activity among the mushroom extract supplemented groups of Px rats. The fraction velocity of glycogen synthase in the soleus and quadriceps muscles was highest in Px rats receiving *C. militaris*, and the velocity increased up to a similar level to the control-Sham group.

Discussion

Px rats were used in this study as an experimental type 2 diabetes model as they well represent a pathophysiology similar to mild type 2 diabetes in this study. Even though total β-cell mass decreases in Px rats, the remaining pancreas has the same proportion of α- and β-cells, and the ratio of serum insulin and glucagons levels is maintained as similar to that of Sham rats.[11)] Px rats are characterized by near-normal or slightly higher fasting blood glucose levels, elevated post-prandial glucose levels, increased insulin resistance, and decreased insulin secretion.[10,20)] To determine the anti-diabetic effects of *P. tenuipes*, *P. linteus*, and *C. militaris* water extracts in elevated insulin resistant states, Px rats were provided with a 40% fat diet and either *P. tenuipes*, *P. linteus*, *C. militaris*, or a placebo. High fat feeding developed impaired intracellular glucose metabolism by suppressing skeletal glycolysis and increasing lipid oxidation. This imparied glucose metabolism was associated with impaired intracellular insulin action.[21)]

Herb water extract including mushrooms contains dietary fiber, and dietary fiber can affect serum glucose levels by altering glucose absorption. To eliminate a possible conflict, cellulose, one kind of dietary fiber, was used as a placebo. But our preliminary study indicated that the hypoglycemic effect of *C. militaris* and *P. tenuipes* water extracts was not due to inhibition of glucose uptake in the gastrointestinal tract (data not shown). Oral administration and intra-peritoneal injection of these extracts or saline showed the similar results in insulin secretion and insulin resistance (unpublished data). Kiho *et al.*[22)] reported that serum glucose levels rapidly decreased and were maintained for 6 h in diabetic (dd) mice when polysaccharides isolated from Cordyceps senensis were intra-peritoneally injected. They also reported that oral administration of Cordyceps senensis-derived polysaccharides also showed hypoglycemic effects, but that the effect was rather less than polysaccharides also showed hypoglycemic effects, but that the effect was rather less than intra-peritoneal injection, indicating that the hypoglycemic effect is not due to inhibition of glucose uptake in the gastrointestinal

tract. Some studies have shown that polysaccharides from mushroom hve α-glucosidase inhibitory effects to decrease or slow down glucose uptake in the gastrointestinal tract. Trehalose from the seeds of Momordica charantia and the fruit bodies of Grifola frondos showed α-glucosidase inhibitory activities *in vitro*.[23)]

Recent research reports that some mushrooms such as Agaricus blazei Murill and Cordyceps senensis have a hypoglycemic effect as well as anti-carcinogenic, antibiotic, and hypocholesterolemic effects.[24,25)] Enhancing the insulin secretion capacity of pancreatic β-cells and the insulin sensitivity of peripheral tissues is important to prevent the prevalence of diabetes and delay diabetes progression. Consumption of Auricularia auricula-judae Quel (30 g/Kg of diet) has been found to lower plasma glucose and insulin, urinary glucose, and food intake in genetically diabetic mice (KK-Ay) from 10 to 14 weeks of age.[26)] The consumption alleviated glucose intolerance to intra-peritoneal glucose loading. In another study, a neutral fraction of Auricularia auricula-judae Quel water extracts showed a hypoglycemic effect, but not the acidic fraction.[27)] Gray and Flatt[28)] found that Agaricus campestris has an antihyperglycemic effect by enhancing insulin-releasing and insulin-like activity. A water extract of Agaricus campestris improved insulin-like activity by stimulating 2-deoxyglucose transport (2.0-fold), glucose oxidation (1.5-fold), and the incorporation of glucose into glycogen (1.8-fold) in muscle of streptozotocin induced diabetic mice. Water mushroom extract evoked a 3.5- to 4.6-fold stimulation of insulin secretion from the BRIN-BD11 pancreatic β-cell line.

P. linteus, *P. tenuipes*, and *C. militaris* have not been studied thoroughly for their medical benefits, since they have not been commercially available until the last 10 years. Most studies reporting on the consumption of mushrooms have shown hypoglycemic effects.[25,27)] The present study indicates that oral administration of *C. militaris* decreases fasting serum glucose levels by increasing glucose disposal rates in Px rats, but in our study, *P. tenuipes* water extract consumption decreased peripheral glucose utilization and insulin secretion capacity. Thus, *P. tenuipes* water extract consumption might be detrimental for type 2 diabetes patients even though *P. tenuipes* and *C. militaris* are caterpillar fungi, that derive nutrients from host bodies while growing in the host insects.

In conclusion, *C. militaris* water extract had beneficial effects on insulin utilization by increasing the glucose disposal rate in skeletal muscles without altering the insulin secretion capacity of pancreatic β-cells. *C. militaris* water extract contains a compound that acts as an insulin sensitizer, and an attempt will be made to isolate it in future studies. But *P. tenuipes* showed rather deleterious effects on treating diabetes; it suppressed the insulin secretion capacity of pancreatic β-cells, whole body glucose disposal in rats, and glucose utilization in skeletal muscles.

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