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Cordyceps militaris alleviates non-alcoholic fatty liver disease in ob/ob mice

Ha-Neul Choi[1], Yang-Hee Jang[1], Min-Joo Kim[2], Min Jeong Seo[3,4], Byoung Won Kang[4], Yong Kee Jeong[3,4] and Jung-In Kim[1§]

[1]Department of Smart Food and Drugs, School of Food and Life Science, Inje University, 197 Inje-ro, Gimhae, Gyeongnam 621-749, Korea
[2]Laboratory of Nutritional Analysis, Hurom Co., Ltd., Gyeongnam 660-701, Korea
[3]Department of Biotechnology, Dong-A University, Busan 604-714, Korea
[4]Medi-Farm Industrialization Research Center, Dong-A University, Busan 604-714, Korea

BACKGROUND/OBJECTIVES: Non-alcoholic fatty liver disease (NAFLD) is becoming an important public health problem as metabolic syndrome and type 2 diabetes have become epidemic. In this study we investigated the protective effect of *Cordyceps militaris* (*C. militaris*) against NAFLD in an obese mouse model.
MATERIALS/METHODS: Four-week-old male ob/ob mice were fed an AIN-93G diet or a diet containing 1% *C. militaris* water extract for 10 weeks after 1 week of adaptation. Serum glucose, insulin, free fatty acid (FFA), alanine transaminase (ALT), and proinflammatory cytokines were measured. Hepatic levels of lipids, glutathione (GSH), and lipid peroxide were determined.
RESULTS: Consumption of *C. militaris* significantly decreased serum glucose, as well as homeostasis model assessment for insulin resistance (HOMA-IR), in ob/ob mice. In addition to lowering serum FFA levels, *C. militaris* also significantly decreased hepatic total lipids and triglyceride contents. Serum ALT activities and tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels were reduced by *C. militaris*. Consumption of *C. militaris* increased hepatic GSH and reduced lipid peroxide levels.
CONCLUSIONS: These results indicate that *C. militaris* can exert protective effects against development of NAFLD, partly by reducing inflammatory cytokines and improving hepatic antioxidant status in ob/ob mice.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of liver diseases, which include hepatic steatosis (fatty liver), non-alcoholic steatohepatitis (NASH), and advanced fibrosis and cirrhosis in the absence of chronic alcohol use [1]. NAFLD is the most common cause of liver disease [2]. Although relatively benign, steatosis can progress to NASH, an extreme form of NAFLD, and NASH can eventually develop into liver cirrhosis [3]. NAFLD has also been suggested to potentiate liver damage induced by other factors, including alcohol, toxins, and viruses [4]. NAFLD prevalence is estimated at 15-40% in Western countries and 9-40% in Asia [5].

Although the underlying mechanism of NAFLD is not clear, the '2-hit hypothesis' was proposed to explain the pathogenesis [3,6]. The 'first hit' is accumulation of triglycerides in the liver (steatosis), which is strongly associated with insulin resistance. The fatty liver is susceptible to injury mediated by the 'second hit', which includes inflammatory adipokines/cytokines, oxidative stress, and mitochondrial dysfunction, leading to steatohepatitis and fibrosis. Insulin resistance is a major pathology underlying the development and progression of NASH [7]. Proinflammatory cytokines such as tumor necrosis factor-α (TNF-a) and interleukin-6 (IL-6) have been suggested to play a crucial role in the development of insulin resistance [8].

Because metabolic syndrome and type 2 diabetes are important risk factors for NAFLD [9], NAFLD is becoming an important public health problem as these have become epidemic [10]. Although numerous therapeutic agents have been postulated to treat NAFLD [11], no pharmacological treatment is to date known [12].

The *Cordyceps* species are entomopathogenic fungi that are used as medicinal mushrooms in eastern Asia [13]. Among them, *Cordyceps sinensis* (*C. sinensis*) is the most valued mushroom, which has pharmacological effects that are used in traditional Chinese medicine. However, natural *C. sinensis* is scarce and highly expensive. Therefore, *Cordyceps militaris* (*C. militaris*) is a prominent substitute for *C. sinensis* due to its similar composition and pharmacological effects to *C. sinensis* and reasonable price [14].

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[§] Corresponding Author: Jung-In Kim, Tel. 82-55-320-3236, Fax. 82-55-321-0691, Email. fdsnkiji@inje.ac.kr
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C. militaris has shown antioxidant activity *in vitro* [15,16]. *C. militaris* extract has free radical scavenging activity and has been reported to have hepatoprotective activity *in vitro* [17]. *C. militaris* extract has alleviated oxidative injury in HepG2 cells induced by *tert*-butyl hydroperoxide (t-BHP) by reducing reactive oxygen species (ROS) generation and thiobarbituric acid reactive substances (TBARS) formation. In addition, *C. militaris* has demonstrated anti-inflammatory activity *in vitro* [18]. A hot water extract of *C. militaris* reduced production of nitric oxide (NO) and secretion of TNF-α and IL-6 induced by lipopolysaccharide (LPS) in macrophages. These findings suggest that *C. militaris* could play a beneficial role in alleviation of NAFLD by improving oxidative stress and reducing inflammation.

However, the beneficial effects of *C. militaris* on NAFLD have not been fully investigated. Therefore, in this study, the protective effect of *C. militaris* against NAFLD was investigated in leptin-deficient ob/ob mice, which show obesity, insulin resistance, and hyperglycemia and are used as an animal model of NAFLD [19-22].

MATERIALS AND METHODS

Preparation of water extract of C. militaris

The fruiting bodies of *C. militaris* were freeze-dried and extracted with 20 times their weight of distilled deionized water for 8 h at 90℃ and filtered [23,24]. The extracted solution was evaporated under vacuum at 80℃ and the residue was lyophilized using a freeze-dryer (yield 33.1%).

Animals and experimental protocol

All animal experiments were approved by the Animal Resource Center at our university (approval no. 2011-44). Four-week-old male C57BL/6-Lep$^{ob/ob}$ mice (n = 16) were obtained from Korea Research Institute of Bioscience and Biotechnology, Ochang, Korea. The mice were housed individually under temperature (24 ± 5℃), humidity (55 ± 5%), and light (12 h light/dark cycle) controlled conditions. After acclimating for 1 week, the animals were randomly divided into two groups. The control group was offered an AIN-93G diet [25], while the *C. militaris* group was fed a diet containing 1% *C. militaris* water extract in place of the Alphacel *ad libitum* for 10 weeks.

Collection of blood and liver samples

At the end of the experiment, the mice were sacrificed by cardiac puncture following an overnight fast. Blood and liver samples were collected and serum was separated by centrifugation of blood samples at 1,500 *g* for 15 min. Serum and liver samples were stored at -70℃ for further analysis.

Measurement of serum glucose and insulin

Serum glucose levels were measured by an enzymatic method using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea). Insulin levels were determined using radioimmunoassay kits (Linco Co., St. Charles, MO, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was estimated by dividing the product of fasting glucose (mg/dL) and insulin levels (ng/mL) by 405 [26].

Measurement of serum free fatty acid (FFA) and hepatic lipids

Serum FFA was measured using an assay kit as described by the manufacturer (Bioassay System, Hayward, CA, USA). To determine hepatic lipids, a portion of the liver tissue was homogenized in saline using a Teflon homogenizer and total lipid was extracted by the method developed by Folch *et al.* [27]. Total lipids of the liver were determined by a gravimetric method. The hepatic triglyceride contents were measured by an enzymatic method using a commercial serum triglyceride assay kit (Asan Pharmaceutical Co. Korea).

Measurement of serum alanine transaminase (ALT) and proinflammatory cytokines

Serum alanine transaminase (ALT) activities were measured spectrophotometrically using a commercially available kit (Youngdong Pharmaceutical Co., Yongin, Korea) in accordance with the manufacturer's instructions. Serum levels of TNF-α, IL-6, and monocyte chemotactic protein-1 (MCP-1) were determined using enzyme-linked immunosorbent assay (ELISA) kits specific for mice (eBioscience, Vienna, Austria).

Measurement of antioxidant parameters in liver

Hepatic TBARS were determined using the method of Ohkawa *et al.* [28]. A portion of the liver tissue was homogenized in 5 volumes of 10 mM sodium phosphate buffer (pH 7.4). To 0.5 mL of the homogenate, a solution composed of 15% trichloroacetic acid (TCA), 0.4% thiobarbituric acid (TBA), and 2.5% HCl (1 mL) was added. The reaction mixture was incubated at 100℃ for 45 min, and then cooled on ice. After centrifugation (1,500*g* for 15 min), the absorbance of the supernatant was measured at 532 nm. TBARS were expressed as nmol malondialdehyde (MDA)/g liver. The glutathione (GSH) level in the liver was quantified by the method of Ellman [29]. A portion of the liver sample was homogenized in 9 volumes of 0.1 mM phosphate buffer (pH 7.4). After centrifugation (10,000*g* at 4℃ for 30 min), the supernatant (0.5 mL) was mixed with 4.5 mL of 5,5-dithiobis-2-nitrobenzonic acid (DTNB) working solution containing 10 mM DTNB and 0.1 M phosphate buffer (pH 8.0; 1:90, v/v). After incubation at room temperature for 15 min, the absorbance was measured at 534 nm. The protein content was measured using the Bradford method [30]. The level of GSH was expressed as nmol/mg protein.

Statistical analyses

The data are expressed as means ± standard error of the mean (SEM). Student's *t*-test was used to identify significant differences between the control and experimental groups, and statistical significance was defined as $P < 0.05$.

RESULTS

Body weight and food intake

Body weight, food intake, and feed efficiency ratio (FER) of the ob/ob control mice and the mice supplemented with 1% *C. militaris* water extract are shown in Table 1. The body weight, weight gain, food intake, and FER of the *C. militaris* group were not significantly different from those of the control group.

Table 1. Body weight, food intake, and feed efficiency ratio in ob/ob mice

	Control	*C. militaris*
Initial body weight (g)	23.9 ± 1.1 ns2)	24.6 ± 1.0
Final body weight (g)	46.7 ± 1.2 ns	45.8 ± 1.1
Weight gain (g/day)	0.325 ± 0.019 ns	0.303 ± 0.016
Food intake (g/day)	4.56 ± 0.10 ns	4.33 ± 0.12
FER1) (%)	7.14 ± 0.42 ns	7.07 ± 0.50

Five-week-old ob/ob mice were fed an AIN-93G diet or a diet containing 1% *C. militaris* water extract *ad libitum* for 10 weeks. Values represent mean ± SEM (n = 8).
1) Feed efficiency ratio (%) = (Body weight gain [g/day]/food intake [g/day]) × 100
2) Not significant

Table 2. Serum glucose and insulin levels and HOMA-IR value in ob/ob mice

	Control	*C. militaris*
Serum glucose (mg/dL)	193.9 ± 11.1	160.3 ± 9.1*2)
Serum insulin (ng/mL)	9.98 ± 0.59 ns3)	9.09 ± 0.46
HOMA-IR1)	4.73 ± 0.28	3.60 ± 0.29**

Five-week-old ob/ob mice were fed an AIN-93G diet or a diet containing 1% *C. militaris* water extract *ad libitum* for 10 weeks. Values represent mean ± SEM (n = 8).
1) HOMA-IR = (fasting glucose levels [mg/dL] × fasting insulin levels [ng/mL])/405
2) * $P < 0.05$, ** $P < 0.01$
3) Not significant

Table 3. Hepatic lipids and serum FFA and ALT activities in ob/ob mice

	Control	*C. militaris*
Liver		
Total lipids (mg/g liver)	257.8 ± 18.7	206.7 ± 14.7*
Triglyceride (mg/g liver)	74.1 ± 6.4	55.4 ± 4.9*
Serum		
FFA (mM)	1.27 ± 0.08	1.05 ± 0.06*
ALT (U/L)	181.4 ± 21.4	126.3 ± 13.8*

Five-week-old ob/ob mice were fed an AIN-93G diet or a diet containing 1% *C. militaris* water extract *ad libitum* for 10 weeks. Values represent mean ± SEM (n = 8).
* $P < 0.05$

Glycemic control

The effects of *C. militaris* on glycemic control and insulin resistance are shown in Table 2. Serum glucose levels were significantly lower in the *C. militaris* group than in the control group ($P < 0.05$). Although insulin levels were not significantly different between the two groups, *C. militaris* supplementation significantly reduced the HOMA-IR value in comparison with the control group ($P < 0.01$).

Hepatic lipids and serum FFA and ALT activities

The hepatic total lipid and triglyceride contents of the *C. militaris* group were reduced by 19.8% and 25.3%, respectively, compared with the control group ($P < 0.05$; Table 3). Serum FFA levels were lower in the *C. militaris* group than in the control group ($P < 0.05$; Table 3). Serum ALT activities were significantly reduced by consumption of *C. militaris* in comparison with the control group ($P < 0.05$).

Serum proinflammatory cytokines

Serum TNF-α levels in the *C. militaris* group were significantly decreased by 17.1% in comparison with the control group ($P < 0.05$; Fig. 1). *C. militaris* supplementation reduced the serum IL-6 by 19.9% in comparison with the control group. Serum MCP-1 levels of the *C. militaris* group tended to be low in comparison with the control group, although the difference was not significant.

Hepatic TBARS and GSH contents

The effects of *C. militaris* on lipid peroxide and GSH concentrations in the liver are shown in Fig. 2. Consumption of *C. militaris* decreased hepatic TBARS by 23.4% and increased GSH levels by 18.7% in comparison with the control group ($P < 0.05$).

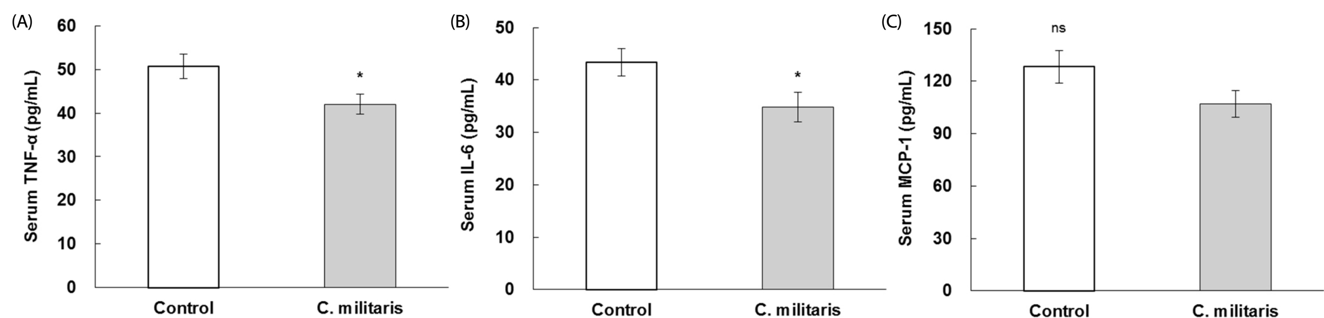

Fig. 1. Serum levels of TNF-α (A), IL-6 (B), and MCP-1 (C) in ob/ob mice. Values represent mean ± SEM (n = 8). * $P < 0.05$. ns Not significant.

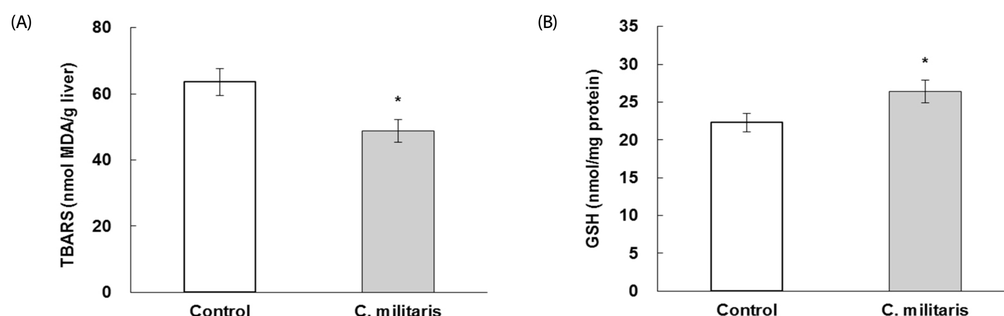

Fig. 2. Hepatic levels of TBARS (A) and GSH (B) in ob/ob mice. Values represent mean ± SEM (n = 8). * $P < 0.05$.

DISCUSSION

We determined the effect of *C. militaris* supplementation on development of fatty liver, oxidative stress, and inflammatory cytokine levels to evaluate its benefit for NAFLD in ob/ob mice. These mice have a mutation in the *ob* gene, which encodes leptin, resulting in hyperphagia and obesity [31]. The hepatocytes of these insulin-resistant mice spontaneously become steatotic, making them a valuable tool for studying NAFLD [32].

Supplementation with 1% *C. militaris* reduced serum glucose and the HOMA-IR, a surrogate parameter of insulin resistance [33] in the ob/ob mice. This finding is in agreement with previous reports. *C. militaris* extract offered at 1% of the diet improved insulin resistance and hyperglycemia without influencing insulin secretion capacity in 90% pancreatectomized rats [34] and in db/db mice [24].

C. militaris extract reduced serum FFA levels in this study. In addition, it decreased hepatic total lipids and triglyceride contents and serum ALT, suggesting alleviation of fatty liver and improvement of liver function. Insulin resistance increases secretion of FFAs from peripheral adipose tissue due to enhanced lipolysis [35], resulting in elevated FFA uptake by the liver, which in turn is converted into triglycerides [36]. Reportedly, FFAs in the blood are elevated in animal models of NAFLD [37] and in NAFLD patients [36].

The improvement in insulin resistance by *C. militaris* may be partially related to its effect on proinflammatory cytokines. *C. militaris* decreased serum TNF-α and IL-6 levels. *C. militaris* also showed a tendency to decrease serum MCP-1, although the difference was not statistically significant. These findings are in agreement with a previous report that *C. militaris* reduced TNF-α and IL-6 secretion by LPS-treated murine macrophages [18]. TNF-α mediates insulin resistance by impairing insulin signal transduction [38]. IL-6 affects insulin signaling, leading to development of insulin resistance [39]. MCP-1 contributes to development of the insulin resistance and hepatic steatosis associated with obesity [40]. Thus, the reduction in TNF-α and IL-6 levels induced by *C. militaris* could contribute to improvement of insulin sensitivity and attenuation of hepatic steatosis.

ob/ob mice with fatty liver have been reported to show increased production of ROS, suggesting increased oxidative stress [41]. Steatotic liver is vulnerable to the 'second hit' mediated by oxidative stress, which leads to inflammation [3,6]. In this study, *C. militaris* reduced hepatic TBARS, suggesting alleviation of the oxidative stress in ob/ob mice. *C. militaris* extract was reported to exert a potent antioxidant activity by directly scavenging free radicals [15,16]. In addition, we demonstrated that *C. militaris* elevated hepatic GSH levels in ob/ob mice. *C. militaris* extract has been shown to increase GSH levels in t-BHP-treated HepG2 cells [17]. Ramesh *et al.* reported that cordycepin (3'-deoxyadenosin), one of the major bioactive components of *C. militaris*, elevated hepatic GSH and reduced TBARS in aged rats, suggesting that cordycepin could be the active component in terms of antioxidant activities [42]. Since nuclear erythroid-related factor 2 (Nrf2) plays a key role in synthesis of GSH [43], further study to investigate the effect of *C. militaris* and cordycepin on Nrf2-signaling pathway could be needed to elucidate the mechanism underlying the antioxidant activity. GSH is an antioxidant involved in scavenging ROS and removing lipid peroxides [44]. Thus, *C. militaris* could contribute to amelioration of the progression of NAFLD by decreasing oxidative stress.

In conclusion, *C. militaris* effectively reduced serum glucose and FFA levels and improved insulin sensitivity in ob/ob mice. *C. militaris* also reduced hepatic triglyceride accumulation and serum ALT. In addition, *C. militaris* ameliorated hepatic oxidative stress and reduced serum proinflammatory cytokine levels. Therefore, *C. militaris* may be beneficial in preventing NAFLD.

REFERENCES

1. Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002;123:1705-25.
2. Petta S, Muratore C, Craxì A. Non-alcoholic fatty liver disease pathogenesis: the present and the future. Dig Liver Dis 2009;41:615-25.
3. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842-5.
4. Salt WB 2nd. Nonalcoholic fatty liver disease (NAFLD): a comprehensive review. J Insur Med 2004;36:27-41.
5. Ahmed MH, Abu EO, Byrne CD. Non-Alcoholic Fatty Liver Disease (NAFLD): new challenge for general practitioners and important burden for health authorities? Prim Care Diabetes 2010;4:129-37.
6. Day CP. From fat to inflammation. Gastroenterology 2006;130:207-10.
7. Marchesini G, Forlani G. NASH: from liver diseases to metabolic disorders and back to clinical hepatology. Hepatology 2002;35:497-9.
8. Tilg H, Moschen AR. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. Trends Endocrinol Metab 2008;19:371-9.
9. Lebovics E, Rubin J. Non-alcoholic fatty liver disease (NAFLD): why you should care, when you should worry, what you should do. Diabetes Metab Res Rev 2011;27:419-24.
10. Angulo P. Obesity and nonalcoholic fatty liver disease. Nutr Rev 2007;65:S57-63.
11. Ibrahim MA, Kelleni M, Geddawy A. Nonalcoholic fatty liver disease: current and potential therapies. Life Sci 2013;92:114-8.
12. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221-31.
13. Paterson RR. Cordyceps: a traditional Chinese medicine and another fungal therapeutic biofactory? Phytochemistry 2008;69:1469-95.
14. Ng TB, Wang HX. Pharmacological actions of Cordyceps, a prized folk medicine. J Pharm Pharmacol 2005;57:1509-19.
15. Yu HM, Wang BS, Huang SC, Duh PD. Comparison of protective effects between cultured Cordyceps militaris and natural Cordyceps sinensis against oxidative damage. J Agric Food Chem 2006;54:3132-8.
16. Zhan Y, Dong CH, Yao YJ. Antioxidant activities of aqueous extract from cultivated fruit-bodies of Cordyceps militaris (L.) Link in vitro. J Integr Plant Biol 2006;48:1365-70.
17. Wang BS, Lee CP, Chen ZT, Yu HM, Duh PD. Comparison of the hepatoprotective activity between cultured Cordyceps militaris and natural Cordyceps sinensis. J Funct Foods 2012;4:489-95.
18. Jo WS, Choi YJ, Kim HJ, Lee JY, Nam BH, Lee JD, Lee SW, Seo SY, Jeong MH. The anti-inflammatory effects of water extract from

Cordyceps militaris in murine macrophage. Mycobiology 2010;38: 46-51.
19. Anstee QM, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. Int J Exp Pathol 2006;87:1-16.
20. Weisberg SP, Leibel R, Tortoriello DV. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology 2008;149:3549-58.
21. Bruno RS, Dugan CE, Smyth JA, DiNatale DA, Koo SI. Green tea extract protects leptin-deficient, spontaneously obese mice from hepatic steatosis and injury. J Nutr 2008;138:323-31.
22. Massart J, Robin MA, Noury F, Fautrel A, Lettéron P, Bado A, Eliat PA, Fromenty B. Pentoxifylline aggravates fatty liver in obese and diabetic ob/ob mice by increasing intestinal glucose absorption and activating hepatic lipogenesis. Br J Pharmacol 2012;165:1361-74.
23. Zhang G, Huang Y, Bian Y, Wong JH, Ng TB, Wang H. Hypoglycemic activity of the fungi Cordyceps militaris, Cordyceps sinensis, Tricholoma mongolicum, and Omphalia lapidescens in streptozotocin-induced diabetic rats. Appl Microbiol Biotechnol 2006;72:1152-6.
24. Choi HN, Kang MJ, Jeong SM, Seo MJ, Kang BW, Jeong YK, Kim JI. Effect of Dongchunghacho (Cordyceps militaris) on hyperglycemia and dyslipidemia in type 2 diabetic db/db mice. Food Sci Biotechnol 2012;21:1157-62.
25. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939-51.
26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
27. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497-509.
28. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
29. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82: 70-7.
30. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
31. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995; 269:543-6.
32. Koteish A, Mae Diehl A. Animal models of steatohepatitis. Best Pract Res Clin Gastroenterol 2002;16:679-90.
33. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008;294:E15-26.
34. Choi SB, Park CH, Choi MK, Jun DW, Park S. Improvement of insulin resistance and insulin secretion by water extracts of Cordyceps militaris, Phellinus linteus, and Paecilomyces tenuipes in 90% pancreatectomized rats. Biosci Biotechnol Biochem 2004;68:2257-64.
35. Jensen MD, Haymond MW, Rizza RA, Cryer PE, Miles JM. Influence of body fat distribution on free fatty acid metabolism in obesity. J Clin Invest 1989;83:1168-73.
36. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005;115:1343-51.
37. Gauthier MS, Couturier K, Latour JG, Lavoie JM. Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. J Appl Physiol (1985) 2003;94:2127-34.
38. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 1994;43:1271-8.
39. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. J Biol Chem 2003;278:13740-6.
40. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 2006;116:1494-505.
41. Yang S, Zhu H, Li Y, Lin H, Gabrielson K, Trush MA, Diehl AM. Mitochondrial adaptations to obesity-related oxidant stress. Arch Biochem Biophys 2000;378:259-68.
42. Ramesh T, Yoo SK, Kim SW, Hwang SY, Sohn SH, Kim IW, Kim SK. Cordycepin (3'-deoxyadenosine) attenuates age-related oxidative stress and ameliorates antioxidant capacity in rats. Exp Gerontol 2012;47:979-87.
43. Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. J Biol Chem 1999;274:33627-36.
44. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother 2003;57:145-55.