

## Improvement on Lipid Metabolic Disorder by 3'-Deoxyadenosine in High-Fat-Diet-Induced Fatty Mice

Ya-Jing Niu,\* Rong-Ya Tao,\* Qian Liu,\* Jin-Ying Tian,\*  
Fei Ye,\* Ping Zhu\*<sup>†</sup> and Hai-Bo Zhu\*

*\*Institute of Materia Medica  
Chinese Academy of Medical Sciences and  
Perking Union Medical College  
Beijing 100050, China*

*<sup>†</sup>Key Laboratory of Biosynthesis of Natural Products  
Ministry of Health of PRC and  
Key Laboratory of Bioactive Substances and  
Resources Utilization of Chinese Herbal Medicine  
Ministry of Education of PCR  
Beijing 100050, China*

**Abstract:** This study explores the effects of 3'-deoxyadenosine, a compound from *Cordyceps militaris*, on lipid metabolic disorder induced by a high-fat-diet in C57BL/6 mice. These mice had an obese body, lipid metabolic disorder and insulin resistance and were treated orally with 100 mg/kg/day 3'-deoxyadenosine (DA), 15 mg/kg/day rosiglitazone and 150 mg/kg/day fenofibrate, respectively. Compared to the model mice, the body weight gain in DA-treated mice were decreased by 66.5%, serum triglyceride and total cholesterol levels were decreased by 20.7% and 16.7%, respectively, and the triglyceride content in the skeletal muscle was reduced by 41.2%. This treatment also had a significant effect on insulin resistance. In DA-treated mice, the serum insulin levels and homeostasis model assessment of the insulin resistance index were decreased by 30% and 46%, respectively, and the areas under the glucose-time curve were depressed by 18% in the insulin tolerance test and by 21.5% in the oral glucose tolerance test. Finally, the value of glucose infusion rates and insulin induced-glucose uptake into the skeletal muscle in the hyperinsulinemic-euglycemic clamp test were increased by 18% and 41%, respectively, compared to those in the model mice. This data suggests that the effects of DA on lipid metabolic disorder induced by a

high-fat-diet may be linked to its improvement on insulin resistance, especially concerning the increase of insulin sensitivity in the skeletal muscle.

*Keywords:* 3'-Deoxyadenosine; Lipid Metabolic Disorder; Insulin Resistance.

## Introduction

Lipid metabolic disorder is a broad term which encompasses both lipidaemia and the imbalance of lipids in the tissues (Chapman *et al.*, 2008; Nestel *et al.*, 2008). A key component of cardiovascular risk, an abnormality in lipid metabolism can cause dyslipidaemia and ectopic fat accumulation in the tissues, such as the skeletal muscle, and is often involved concomitantly in the clinical context of obesity and insulin resistance (Shen *et al.*, 2003; Grundy, 1998). Insulin resistance is defined as an inadequate response to the physiologic effects of circulating insulin by insulin target tissues. It is a major component of metabolism and a key factor in the etiology of a number of diseases, such as atherosclerosis and type 2 diabetes (Semenkovich, 2006).

The obese and insulin resistant C57BL/6 model mice that developed significant central obesity, insulin resistance, impaired glucose tolerance and dyslipidemia while on a chronic high-fat-diet are a valuable tool for the mechanistic investigation of metabolic syndrome and the evaluation of therapeutic effects of related drugs (Almind and Kahn, 2004; Biddinger *et al.*, 2005). All of these characteristics of metabolic syndrome are solely induced by increased nutrition in *ad libitum* condition. High-fat-diet induced obese and insulin resistant mice are an excellent model for the study of the natural pathogenesis of metabolic syndrome as it occurs in clinic.

Some natural products have been shown to help treat this disease and it may be beneficial to find candidates for these product's leading active compounds, since their chemical structure might differ from those of well-known commercial hypolipidemic medicines. It has been reported that 3'-deoxyadenosine (DA), also known as *Cordycepin* and found in *Cordyceps militaris*, is used as a tonic herb in traditional Chinese medicine (Choi *et al.*, 2004; Chang *et al.*, 2008). DA has many pharmacological activities, such as anti-cancer, anti-virus and anti-infection activities and immunological stimulation (Choi *et al.*, 2004). Recently, the remarkable hypolipidemia function of DA has been found (Zhu *et al.*, 2003). In this study, the ability of DA in treating lipid metabolic disorder, especially triglyceride accumulation in the skeletal muscle, was explored, and its synchronal effects on insulin resistance were investigated.

## Materials and Methods

### *Agent*

The compound 3'-deoxyadenosine (DA) was isolated, purified, and identified from the cultured fruiting body of *Cordyceps militaris* by the main laboratory of biosynthesis of Natural Products, Ministry of Health of PRC in Institute of Metraia Medica, Chinese

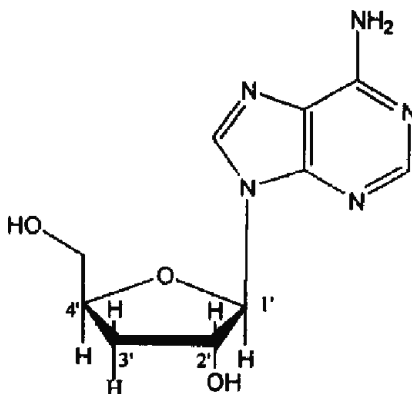


Figure 1. The chemical structure of DA.

Academy of Medical Sciences & Peking Union Medical College. The chemical structure of DA is shown in Fig. 1.

The extraction and purification of cordycepin: 1 kg of the cultured fruiting body of *Cordyceps militaris* was soaked in ethanol overnight and extracted by thermal recycling extraction 3 times for 3–4 hours each. The extract solution was concentrated under reduced pressure to dryness. The extract was dissolved in alcohol, mixed with silica gel, evaporated to dryness, and baked for 3–4 hours at 60°C. DA was separated by a GF254 SIL column (9 cm × 60 cm) with the mobile phase of petroleum ether/dichloromethane/methanol (1.5:3:0.6). Samples were collected (500 ml of each fraction) and traced by TLC, with the authentic substance 3'-deoxyadenosine (Sigma). The fraction with cordycepin was concentrated under reduced pressure to dryness and recrystallized with ethanol. Light yellow crystal was collected and identified as 3'-deoxyadenosine (DA) by chromatographic analysis (MS, NMR) and elemental analysis with a purity of > 99% (HPLC).

### Materials

Fenofibrate and rosiglitazone were obtained from Laboratoires Fournier S.A. (Chenove, France) and GlaxoSmithKline (USA), respectively. The fluorescent compound 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) was purchased from Invitrogen Corporation (Carlsbad, CA, USA). Commercial kits for the measurement of triglyceride (TG) and total cholesterol (TC) concentrations were obtained from Zhongsheng Beikong Bio-Technology and Science (Beijing, China). Insulin was purchased from Novo Nordisk (Denmark); sodium pentobarbital, glucose, and other reagents were purchased from the Beijing Chemical Reagents Company (Beijing, China).

### Biochemical Assay

The blood glucose level was determined by the glucose analyzer (Biosen 5030, EKF Diagnostic, Germany). The protein concentration was measured by the Bio-Rad protein

assay reagent (Bio-Rad, Hercules, CA, USA) with a microplate spectrophotometer ( $\mu$ Quant, Bio-Tek Instruments, Inc., USA). Serum triglyceride and cholesterol levels were determined according to the kit instructions. Muscle triglyceride content was determined from glycerol residues after extraction and separation of the muscle samples according to the reported methods (Turcotte *et al.*, 1999). The serum insulin level was estimated by a radioimmunoassay kit obtained from the Northern Bioengineering Institute (Beijing, China) using a liquid scintillation and luminescence counter (1450 MicroBeta, Perkin-Elmer, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated by the formula ( $\text{HOMA-IR} = \text{serum insulin levels} \times \text{blood glucose levels} / 22.5$ ). White adipose tissue included epididymal fat pad and abdominal adipose tissue. Adiposity index was calculated as white adipose tissue weight (g)/body weight (g)  $\times 100$  (Cong *et al.*, 2008).

#### *Animals and Treatment*

Four-week-old male C57BL/6J mice were obtained from the Animal Center of the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College. The animals were housed in a room with a temperature of 21–23°C and 40–60% humidity. They were exposed to a 12-hours lighting cycle (06:00–18:00 light, 18:00–06:00 dark) and were allowed *ad libitum* access to water and the appointed food source. The animal models were induced by a high-calorie diet for 20 weeks according to the method previously described (Cong *et al.*, 2008). The animals which showed obesity and exhibited a smaller change in insulin-induced blood glucose in the insulin resistance test (ITT) were chosen as the insulin-resistance model and divided randomly into four groups: model control (IRF), insulin sensitizer rosiglitazone 15 mg/kg (Rosi), lipid metabolism regulator fenofibrate 150 mg/kg (Feno) and DA 100 mg/kg (DA). All the drugs were dissolved in water and given by gavage once a day. The IRF group received the same volume water by gavage once a day. Age-matched male C57BL/6J mice fed the standard laboratory food were used as normal controls (Con). All experimental procedures were approved by the Animal Care and Use Committee in Beijing, China.

#### *Oral Glucose Tolerance Test (OGTT)*

The mice were orally administered 2 g/kg glucose after having fasted for 2 hours. Blood samples were collected from the tails at 0, 30, 60, and 120 min after glucose loading to determine the glucose concentrations. The values of area under the glucose-time curve (AUC) were calculated.

#### *Insulin Tolerance Test (ITT)*

The mice fasted for 2 hours and were then injected with insulin (0.4 U/kg) subcutaneously. Blood samples were collected from the tails at 0, 30, 60, and 120 min after the insulin loading to determine the glucose concentrations. The values of AUC were then calculated.

### *Hyperinsulinemic-Euglycemic Clamp*

The experimental process was performed as described previously, (Ye *et al.*, 2008) with some modification. After fasting for 4–5 hours, the mice were anesthetized with 80 mg/kg sodium pentobarbital (i.p.) and heparinized with 1 U/kg heparin (i.v.). An indwelling catheter was inserted into the left internal jugular vein to allow for the infusion of insulin and glucose and another indwelling catheter was placed in the right internal jugular artery for taking blood samples. Insulin was infused by a programmable syringe pump (Cole Parmer, Vernon Hills, IL, USA), and glucose was perfused by a low-flow, high-accuracy pump (IPC, Ismatec, Switzerland). The hyperinsulinemic-euglycemic clamp technique was carried out with a prime-continuous infusion of human insulin at the rate of 60 pmol/kg/min. Blood samples (10  $\mu$ l) were collected at 10-min intervals for the immediate measurement of blood glucose concentration, which was regulated to the basal level ( $95 \pm 5$  mg/dl) by the perfusion of 10% glucose at variable rates. When the blood glucose had maintained a steady state for at least 20 min, the glucose infusion rate (GIR) was measured three times and averaged. Then, 2-NBDG (250  $\mu$ g/mouse) was injected as a bolus in 1 min. Forty five minutes after the injection, the mice were sacrificed quickly by exsanguination. The liver, epididymal adipose tissue, and skeletal muscle were taken immediately, homogenized in 0.9% sodium chloride, and analyzed for 2-NBDG. The fluorescence intensity and protein concentration in these tissues was determined, and the concentration of 2-NBDG was calculated according to the standard curve of the fluorescence intensity-2-NBDG concentrations.

### *Statistical Analyses*

The one-way ANOVA analysis (SPSS version 11.0, USA) was performed to assess data differences among the groups.  $p < 0.05$  was considered statistically significant.

## **Results**

### *The Effects of DA on Body Weight*

Compared to those in the control group, the model mice fed with a high-fat-diet were significantly obese, as shown by the increase the body weights ( $50.4 \pm 3.4$  g vs.  $31.9 \pm 2.9$  g,  $p < 0.001$ ), the weights of white adipose tissues ( $3.1 \pm 0.6$  g vs.  $1.1 \pm 0.3$  g,  $p < 0.001$ ) and the values of the adiposity index ( $6.1 \pm 1.0$  g vs.  $3.4 \pm 0.9$  g,  $p < 0.001$ ). After DA treatment for 7 weeks, the body weight gain was markedly reduced by 66.5% in the DA-treated group compared to those in IRF group (Table 1). However, the amount of food intake was almost the same among all high-fat-diet groups (data not shown).

### *The Effects of DA on Serum TC and TG Levels*

After DA treatment for 5 weeks, the IRF group demonstrated hypercholesterolemia compared to the control group. The serum TC levels in DA and fenofibrate-treated groups were

**Table 1. The Effects of DA on Lipid Metabolic Profiles**

	Body Weight Gains (g)	Serum TC (mg/dl)	Serum TG (mg/dl)	TG Content in Skeletal Muscles (mg/g Protein)
<b>Con</b>	-0.4 ± 0.9	42.1 ± 9.8	72.4 ± 14.1	44.6 ± 15.8
<b>IRF</b>	2.2 ± 1.6###	100.6 ± 13.8###	81.2 ± 16.0	118.1 ± 56.0###
<b>DA</b>	0.7 ± 1.1*	83.8 ± 14.0*	64.5 ± 9.7**	69.4 ± 24.9*
<b>Feno</b>	-8.0 ± 3.1***	85.7 ± 7.1**	43.4 ± 7.3***	26.3 ± 5.6***
<b>Rosi</b>	8.6 ± 2.9***	106.9 ± 11.0	56.8 ± 5.7***	241.8 ± 92.3***

*Note:* Animals were treated with DA, fenofibrate, or rosiglitazone for 7 weeks. Data is shown as means ± SEM. n = 10. ###, p < 0.001 vs. Con. \*, \*\*, \*\*\*, p < 0.05, 0.01, 0.001 vs. IRF, respectively.

decreased by 16.7% and 14.8%, respectively, compared to those in the IRF group (Table 1). However, there was no difference between the serum TC levels in the IRF group and the Rosi group.

Compared to the IRF group, the serum TG levels in DA, fenofibrate and rosiglitazone-treated groups were decreased significantly by 20.7%, 46.6% and 30.1%, respectively, although serum TG levels in the IRF group were similar to those in the control mice (Table 1).

#### *The Effects of DA on Lipid Accumulation in the Skeletal Muscles*

The triglyceride concentration in the skeletal muscles in the IRF group exhibited by an 165% increase compared to that in the normal control group. Triglyceride concentration in the skeletal muscles of DA and fenofibrate-treated groups was significantly decreased by 41.2% and 77.7% respectively (Table 1). However, compared to that in the IRF group, triglyceride concentration in the skeletal muscle of the rosiglitazone-treated group was significantly increased by 104.8%.

#### *The Effects of DA on Insulin Sensitivity*

The insulin resistance was induced by a high-fat-diet in male C57BL/6 mice and can be reversed by DA (Figs. 2 and 3). The response to glucose loading after the treatment for 6 weeks is shown in Fig. 2. After the glucose loading in OGTT, the peak of blood glucose levels was significantly increased by 121% and the AUC value was also significantly increased by 41.2% in the IRF group. Compared to those in the IRF group, the peak of blood glucose levels and the AUC values in the DA group were decreased by 21% and 22%, respectively. Similar results were also observed in both the rosiglitazone- and the fenofibrate-treated groups (Figs. 2A, 2B).

The response to insulin loading after the treatment for 4 weeks was investigated. After the insulin loading in ITT, the AUC values in the IRF group were higher by 1.64 fold than those in the control group. The results showed that the DA treatment improved insulin resistance by significantly decreasing blood glucose after insulin loading, and a marked reduction of AUC values by 17.6%, compared to the IRF group. These effects were similar to the results from the rosiglitazone and fenofibrate treatments (Figs. 2C, 2D).

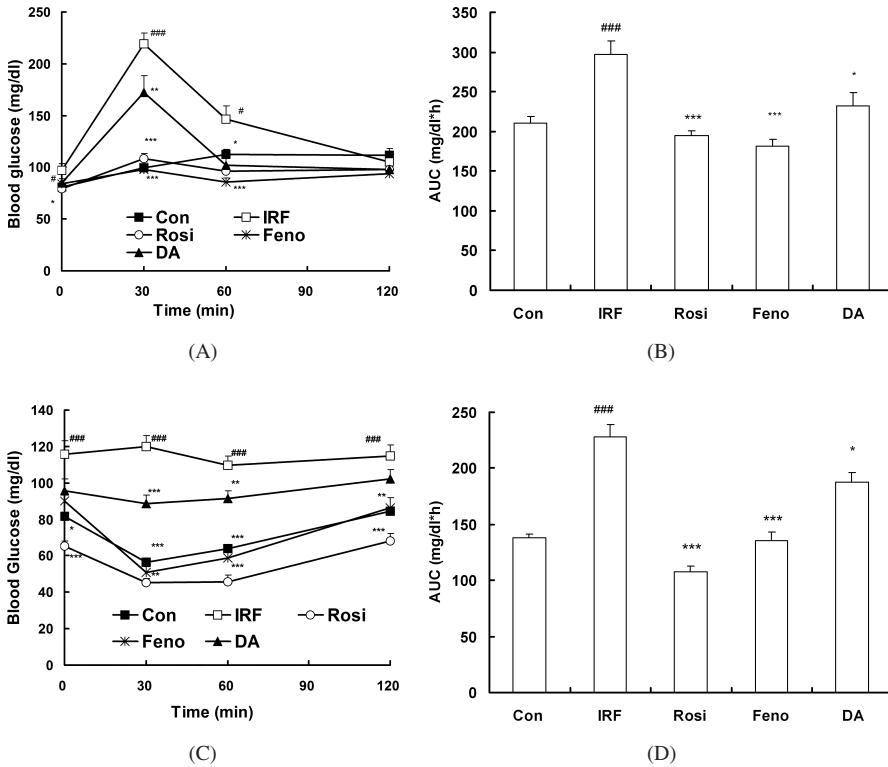


Figure 2. The effects of DA on glucose and insulin tolerance in mice. The glucose tolerance test was performed after the animals treated for 46 days. The insulin tolerance test was performed after the animals treated for 29 days. (A) Changes of blood glucose in OGTT; (B) AUC values in OGTT; (C) changes of blood glucose in ITT; (D) AUC values in ITT. n = 10. #, ##, ###, p < 0.05, 0.01, 0.001 vs. Con respectively. \*, \*\*, \*\*\*, p < 0.05, 0.01, 0.001 vs. IRF, respectively.

Compared to those in the normal control group, the levels of HOMA-IR index and serum insulin in the IRF group were increased by 11.6 and 10.3 fold, respectively. Compared to those in the IRF group, the index of HOMA-IR and the level of serum insulin in the DA-treated group, after 7 weeks of treatment, were markedly decreased by 46% and 30%, respectively. Similar results were observed in the groups treated with rosiglitazone and fenofibrate (Figs. 3A, 3B).

The hyperinsulinemic-euglycemic clamp test was performed after 4 weeks of treatment. The value of GIR in response to the high insulin clamp was 7.2-fold lower in the IRF group than that in the control mice, and, as described previously, the 2-NBDG uptake into the liver, adipose tissue, and skeletal muscles was 43.3%, 63.9%, and 36.6% lower, respectively than those in the control mice (Ye *et al.*, 2008) (data not shown). Compared to those in IRF group, the value of GIR was increased by 377.0% and the 2-NBDG uptake into the liver, white adipose, and skeletal muscle was enhanced by 157%, 42%, and 140% respectively, in the group treated with rosiglitazone. In the group treated with DA, the value of GIR was markedly increased by 18% (p < 0.032), and the 2-NBDG uptake into

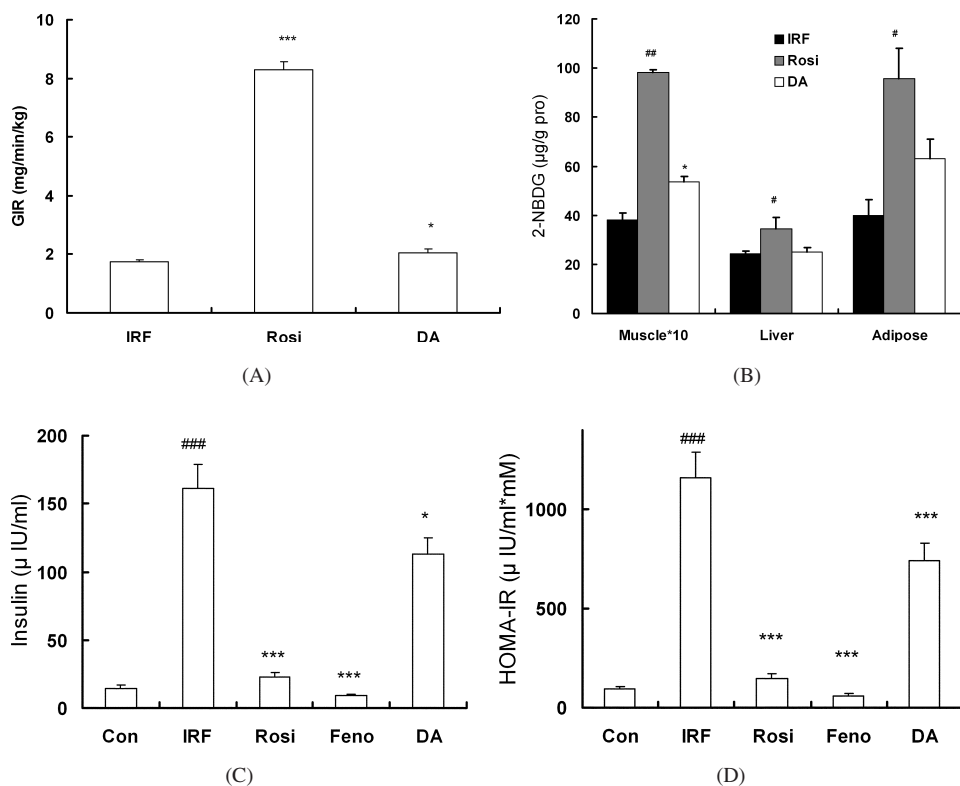


Figure 3. The effects of DA on insulin resistance. Animals were treated with DA, rosiglitazone and fenofibrate for 36 days. (A) The values of GIR in hyperinsulinemic-euglycemic clamp test; (B) the efficacy of insulin-induced glucose uptake; (C) the levels of serum insulin levels; (D) the index of HOMA-IR.  $n = 10$ . #, ##, ###,  $p < 0.05$ , 0.01, 0.001 vs. Con respectively. \*, \*\*, \*\*\*,  $p < 0.05$ , 0.01, 0.001 vs. IRF respectively.

the skeletal muscles was increased by 41% ( $p = 0.032$ ). However, there were no significant changes of 2-NBDG uptake into the liver and white adipose in the DA-treated group (Figs. 3C, 3D).

## Discussion

Natural products are becoming the major molecular structural resources for drug discovery. *Cordyceps militaris* is a traditional medicinal mushroom, well known for its immunostimulatory and anticancer activities (Jin *et al.*, 2008; Hubbell *et al.*, 1985; Nan *et al.*, 2001). It is usually used to treat hemoptysis, bronchial or lung inflammation, and urogenital disorders (Jin *et al.*, 2008). However, wild *Cordyceps sinensis* is rare and very difficult to culture artificially. On the other hand, *Cordyceps militaris*, another species of *Cordyceps*, is widely used in Southeast Asia and China at present. DA (3'-deoxyadenosine) is an adenosine analog, extracted from *Cordyceps militaris*, and has been used as an anticancer and anti-inflammation ingredient in traditional Chinese medicine. Its antitumor effects on the



cancers of bladder, colon, and lungs, as well as fibrosarcoma, and its anti-inflammatory effects regarding the production of inflammatory mediators have been reported (Hubbell *et al.*, 1985; Nan *et al.*, 2001). The inhibitory effects of DA on the adenylate cyclase activity in both particulated platelet fraction and platelet membrane (Haslam *et al.*, 1978; Cho *et al.*, 2007) and its remarkable hypolipidemia function have also been found (Zhu *et al.*, 2003). This study investigated the effects of DA on lipid metabolic disorder and the way in which it fights the disease.

Lipid metabolic disorder has been found to play an important role in type 2 diabetes (Schenk *et al.*, 2008). The increase in the intracellular deposition of TG in the muscles, liver and pancreas in subjects prone to diabetes is well documented (Raz *et al.*, 2005). A hallmark of type 2 diabetes, intramyocellular lipid accumulation has been associated with insulin resistance in humans (Krssak *et al.*, 1999) and animals (Korach-Andre *et al.*, 2005; Kuhlmann *et al.*, 2005). Therefore, intramyocellular lipid accumulation can serve as a biomarker of impaired insulin resistance and can potentially be used to test compound efficacy and the amelioration of the insulin resistant state (Kuhlmann *et al.*, 2005). In this study, DA inhibited the body weight gains in high-fat-diet induced fatty mice. DA also had an effect on the serum TG levels and TG accumulation in the skeletal muscles. However, compared to DA, rosiglitazone can increase the TG accumulation in the skeletal muscles of C57BL/6 mice on a high-fat-diet. At the same time, fenofibrate can increase the liver weight of C57BL/6 mice on a high-fat-diet, perhaps due to its side effect.

Insulin resistance is a key player in both dyslipidaemia and the pathophysiology of metabolic syndrome and has even been postulated to be the underlying cause of the latter (Biddinger *et al.*, 2005; Grundy *et al.*, 1998). Compared to the model group, DA can ameliorate oral glucose tolerance and insulin tolerance, and increase the insulin-dependent glucose uptake in skeletal muscles. These results indicate that DA improves insulin sensitivity and glucose intolerance.

Skeletal muscles are a major peripheral tissue that accounts for approximately 40% of the total body mass and is a major player in energy balance (Smith and Muscat, 2005). Skeletal muscles have also been identified as the major tissue involved in glucose metabolism, accounting for more than 75% of whole-body insulin-stimulated glucose uptake (Shulman, 1990). In this study, DA increased the insulin-dependent glucose uptake into the skeletal muscle and lowered the TG accumulation in them. These results suggest that skeletal muscles may be the major target tissue of DA.

In conclusion, the effects of DA, a compound from *Cordyceps militaris*, on lipid metabolic disorder in high-fat-fed C57BL/6 mice were investigated and its main function may be improved insulin resistance, especially in the skeletal muscles. Thus, DA has the potential to be developed as a drug for the treatment of lipid metabolic disorder.

## Acknowledgments

This work is supported by the Natural Sciences Funds of China (No. 30572215) and National S&T Major Project (No. 2009ZX09103-432; 2009ZX09303-003).

## References

- Almind, K. and C.R. Kahn. Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice. *Diabetes* 53: 3274–3285, 2004.
- Biddinger, S.B., K. Almind, M. Miyazaki, E. Kokkotou, J.M. Ntambi and C.R. Kahn. Effects of diet and genetic background on sterol regulatory element-binding protein-1c, stearoyl-CoA desaturase 1, and the development of the metabolic syndrome. *Diabetes* 54: 1314–1323, 2005.
- Chang, Y., K.C. Jeng, K.F. Huang, Y.C. Lee, C.W. Hou, K.H. Chen, F.Y. Cheng, J.W. Liao and Y.S. Chen. Effect of *Cordyceps militaris* supplementation on sperm production, sperm motility and hormones in Sprague-Dawley rats. *Am. J. Chin. Med.* 36: 849–859, 2008.
- Chapman, M.J. and A.C. Sposito. Hypertension and dyslipidaemia in obesity and insulin resistance: pathophysiology, impact on atherosclerotic disease and pharmacotherapy. *Pharmacol. Ther.* 117: 354–373, 2008.
- Cho, H.J., J.Y. Cho, M.H. Rhee, H.S. Kim, H.S. Lee and H.J. Park. Inhibitory effects of cordycepin (3'-deoxyadenosine), a component of *Cordyceps militaris*, on human platelet aggregation induced by thapsigargin. *J. Microbiol. Biotechnol.* 17(7): 1134–1138, 2007.
- Choi, S.B., C.H. Park, M.K. Choi, D.W. Jun and S. Park. 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.* 68: 2257–2264, 2004.
- Cong, W., R. Tao, J. Tian, G. Liu and F. Ye. The establishment of a novel non-alcoholic steatohepatitis model accompanied with obesity and insulin resistance in mice. *Life Sci.* 82: 983–990, 2008.
- Grundy, S.M. Cholesterol management in high-risk patients without heart disease. When is lipid-lowering medication warranted for primary prevention? *Postgrad. Med.* 104(5): 117–1120, 1998.
- Haslam, R.J., M.M. Davidson and J.V. Desjardins. Inhibition of adenylate cyclase by adenosine analogues in preparations of broken and intact human platelets. Evidence for the unidirectional control of platelet function by cyclic AMP. *Biochem. J.* 176: 83–95, 1978.
- Hubbell, H.R., E.C. Pequignot, D.H. Willis, C. Lee and R.J. Suhadolnik. Differential antiproliferative actions of 2',5' oligo A trimer core and its cordycepin analogue on human tumor cells. *Int. J. Cancer* 36: 389–394, 1985.
- Jin, C.Y., G.Y. Kim and Y.H. Choi. Induction of apoptosis by aqueous extract of *Cordyceps militaris* through activation of caspases and inactivation of Akt in human breast cancer MDA-MB-231 cells. *J. Microbiol. Biotechnol.* 8(12): 1997–2003, 2008.
- Korach-Andre, M., R. Deacon, M. Beil, D. Sun, J. Gao and D. Laurent. Age and muscle-type modulated role of intramyocellular lipids (IMCL) in the progression of insulin resistance in non-diabetic Zucker rats. *Metabolism* 54: 522–528, 2005.
- Krssak, M., P.K. Falk, A. Dresner, L. DiPietro, S.M. Vogel, D.L. Rothman, M. Roden and G.I. Shulman. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42: 113–116, 1999.
- Kuhlmann, J., C. Neumann-Haefelin, U. Belz, W. Kramer, H.P. Juretschke and A.W. Herling. Correlation between insulin resistance and intramyocellular lipid levels in rats. *Mag. Res. Med.* 53: 1275–1282, 2005.
- Nan, J.X., E.J. Park, B.K. Yang, C.H. Song, G. Ko and D.H. Sohn. Antifibrotic effect of extracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rats. *Arch. Pharm. Res.* 24(4): 327–332, 2001.
- Nestel, P.J., R. O'Brien and M. Nelson. Management of dyslipidaemia-evidence and practical recommendations. *Aust. Fam. Physician* 37(7): 521–527, 2008.

- Raz, I., R. Eldor, S. Cernea and E. Shafir. Diabetes: insulin resistance and derangements in lipid metabolism. Cure through intervention in fat transport and storage. *Diabetes Metab. Res. Rev.* 21(1): 3–14, 2005.
- Schenk, S., M. Saberi and J.M. Olefsky. Insulin sensitivity: modulation by nutrients and inflammation. *J. Clin. Invest.* 118(9): 2992–3002, 2008.
- Semenkovich, C.F. Insulin resistance and atherosclerosis. *J. Clin. Invest.* 116(7): 1813–1822, 2006.
- Shen, B.J., J.F. Todaro, R. Niaura, J.M. McCaffery, J. Zhang, A. Spiro and K.D. Ward. Are metabolic risk factors one unified syndrome? Modeling the structure of the metabolic syndrome X. *Am. J. Epidemiol.* 157: 701–711, 2003.
- Shulman, G.I. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin dependent diabetes by <sup>13</sup>C nuclear magnetic resonance spectroscopy. *N Engl. J. Med.* 322: 223–228, 1990.
- Smith, A.G. and G.E. Muscat. Skeletal muscle and nuclear hormone receptors: implications for cardiovascular and metabolic disease. *Int. J. Biochem. Cell Biol.* 37: 2047–2063, 2005.
- Turcotte, L.P., J.R. Swenberger, M.Z. Tucker and A.J. Yee. Training-induced elevation in FABPpm is associated with increased palmitate use in contracting muscle. *J. Appl. Physiol.* 87: 285–293, 1999.
- Ye, F., R. Tao, W. Cong, J. Tian and Q. Liu. Utilization of fluorescence tracer in hyperinsulinemic-euglycemic clamp test in mice. *J. Biochem. Biophys. Methods* 70(6): 978–984, 2008.
- Zhu, P., H.B. Zhu, L. Zhu, L. Zhang, H.X. He, Q. Wang, K.D. Cheng, J.G. Shi and Y.C. Yang. Utilization of 3'-deoxyadenosine in the preparation of hypolipidemic drugs. Chinese Patent ZL 200310101650.7, 2003.

Copyright of American Journal of Chinese Medicine is the property of World Scientific Publishing Company and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.