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Adiponectin Decreases Plasma Glucose and Improves Insulin Sensitivity in Diabetic Swine

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Abstract To investigate the effects of recombinant human adiponectin on the metabolism of diabetic swine induced by feeding a high-fat/high-sucrose diet (HFSD), diabetic animal models were constructed by feeding swine with HFSD for 6 months. The effects of recombinant adiponectin were assessed by detecting the change of plasma glucose levels by commercially available enzymatic method test kits and evaluating the insulin sensitivity by oral glucose tolerance test (OGTT). About 1.5 g purified recombinant adiponectin was produced using a 15-liter fermenter. A single injection of purified recombinant human adiponectin to diabetic swine led to a 2- to 3-fold elevation in circulating adiponectin, which triggered a transient decrease in basal glucose level (P<0.05). This effect on glucose was not associated with an increase in insulin level. Moreover, after adiponectin injection, swine also showed improved insulin sensitivity compared with the control (P<0.05). Adiponectin might have the potential to be a glucose-lowering agent for metabolic disease. Adiponectin as a potent insulin enhancer linking adipose tissue and glucose metabolism could be useful to treat insulin resistance.

Key words adiponectin; insulin sensitivity; diabetic swine

Adiponectin plays an important role in the regulation of energy homeostasis and glucose and lipid metabolism [1–3]. Decreased serum levels of adiponectin is a common feature of obesity and diabetes. It is also correlated with lowered indices of insulin sensitivity in humans and rodents [4–7]. These facts lead to the hypothesis that a decreased serum adiponectin level contributes *in vivo* to insulin resistance. It was further discovered that adiponectin replenishment ameliorates insulin resistance in various models of genetic diabetic mice and rats [8]. The remarkable increase in adiponectin levels in response to treatment with the antidiabetic, insulin-sensitizing agents thiazolidinediones further emphasizes the potential insulin-sensitizing effects of adiponectin [9]. However, the complete physiological role of human adiponectin and its actions *in vivo* are still unclear.

Type 2 diabetes is prevalent in China, and will continue to pose a serious public health problem in the future. Research indicates that there will be 70 million people with diabetes in 2025. Lack of suitable large animal models has hindered the research of diabetes, particularly in the study of type 2 diabetes and the accompanying complications. Swine have many characteristics similar to humans that make them a suitable species to model human diseases.

We investigated the effect of recombinant human adiponectin on insulin and glucose metabolism in diabetic swines. Our results showed that elevated levels of circulating adiponectin enhanced glucose profiles and improved insulin sensitivity in high-fat/high-sucrose diet (HFSD)-induced diabetic swines.

Materials and Methods

Materials

Cloning and expression vector (pET-adiponectin) and

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Escherichia coli BL21-DE3 host strain were stored in our own laboratory. The experimental animals of Chinese Guizhou swine were obtained from the barrier unit at the Laboratory Animal Center of Chongqing Medical University (Chongqing, China). Glucose test kits were from Shanghai Rongsheng Biotech (Shanghai, China). Insulin radioimmunology kits were purchased from the China Institute of Atomic Research (Beijing, China). All other chemicals were of analytical grade.

Recombinant protein production and protein characterization

Recombinant human adiponectin was produced as described in Hu *et al*. [10] except that a 15-liter fermenter was used to produce protein on a large scale. To detect adiponectin, the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose paper by standard procedures. Antibody raised against the globular head domain of adiponectin (gadiponectin) was incubated, then horseradish peroxidase (HRP)-conjugated anti-rabbit IgG was used to visualize the proteins.

Animals and diets

Chinese Guizhou swines were housed in single pens under controlled conditions (temperature, between 18 ºC and 22 ºC; relative air humidity, 30%–70%; air changed four times per hour) and fed twice daily on a restricted schedule with HFSD. The HFSD used in this study was normal swine diet supplemented with 10% lard and 37% sucrose. The total study period was five months. Blood samples were obtained without sedation by pricking an ear vein with a lancet and collecting drops in a hematocrit tube [11].

Plasma measurement

Glucose was determined by a commercially available enzymatic method test kit. Plasma insulin was determined by radioimmunoassay (RIA) using an insulin radioimmunology kit.

Oral glucose tolerance test (OGTT)

After fasting for 18 h (overnight), the HFSD fed animals were offered a glucose tolerance test mixed meal of 25 g swine fodder and 2 g glucose per kilogram of body weight. The meal was eaten from a bowl under supervision. Blood samples were obtained without anesthesia from the orbital sinus at time points of 0, 30, 60, 90, 120, 150, and 180 min relative to the glucose load [11].

Results

Expression and purification of recombinant fusion adiponectin in a 15-liter fermenter

E. coli BL21(DE3) cells transformed with pET-adiponectin were cultured for about 8 h, and was induced by isopropylthio-β-*D*-galactoside (IPTG) for 3 h thereafter. About 530 g bacteria was harvested in total. SDS-PAGE analysis revealed that recombinant adiponectin (Trx-adiponectin) accounted for about 34% of the total proteins of *E. coli*, with an expected molecular mass of 42 kDa. The fusion protein was purified with single-step affinity chromatography on a nickel affinity resin column. After elution, the free recombinant protein was obtained, and the yield was about 100 mg per liter of induced culture (**Fig. 1**).

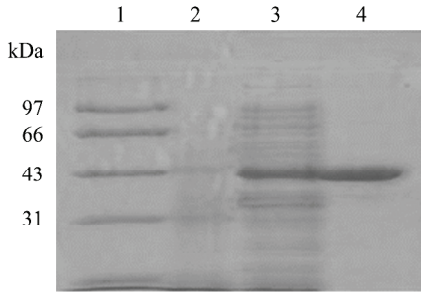

Fig. 1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of adiponectin

1, protein molecular mass marker; 2, protein extracted from *Escherichia coli* transformed with pET-adiponectin but without isopropylthio-β-*D*-galactoside induction; 3, protein extracted from *E. coli* transformed with pET-adiponectin after isopropylthio-β-*D*-galactoside induction; 4, purified recombinant adiponectin fusion protein.

HFSD-induced diabetes

HFSD feeding resulted in a faster increase in body weight than that observed in the control group (**Fig. 2**). Glucose levels increased significantly in HFSD feeding swine. Plasma fasting insulin levels in this group also increased, although not significantly, over the first three months then decreased (**Table 1**). This pattern of fasting plasma insulin response to HFSD feeding would represent induction of insulin resistance followed by a progressive decline in pancreatic β-cell function. Pigs from the HFSD group showed significantly increasing total

Table 1 Effects on glucose, insulin, total cholesterol (TC), triglycerides (TG), and free fatty acids (FFA) in swine fed a high-fat/high-sucrose diet (HFSD)

		0 month	2 months	3 months	4 months	5 months
Glucose (mM)	Control	3.94±0.78	4.51±1.17	4.05±0.56	4.51±1.56	4.65±0.79
	HFSD	3.55±1.10	6.72±0.58 *	6.98±0.98 *	7.50±1.21 *	7.02±0.54 *
Insulin (U/L)	Control	10.71±2.56	10.37±3.05	11.34±3.78	12.67±3.34	11.56±4.02
	HFSD	11.70±3.09	15.83±4.01	16.95±3.08	12.73±2.24	12.79±2.98
TC (mM)	Control	2.28±0.43	1.97±0.60	2.15±0.54	2.07±0.46	2.01±0.63
	HFSD	1.96±0.41	3.74±0.67 *	3.35±0.90 *	3.86 ± 0.95 *	4.16±1.22 *
TG (mM)	Control	0.53±0.12	0.61±0.09 *	0.62±0.12	0.64±0.19	0.64±0.30
	HFSD	0.55±0.16	1.42±0.20 *	1.98±0.42 *	1.98±0.24 **	1.99±0.18 **
FFA (mM)	Control	1.13±0.21	1.10±0.34	0.99±0.28	0.90±0.28	0.82±0.32
	HFSD	1.07±0.23	2.24±0.58 *	1.98±0.60 *	1.98±0.49 *	1.20±0.34

Data are expressed as mean±SD (n=5). *P<0.05 and **P<0.01 vs. control.

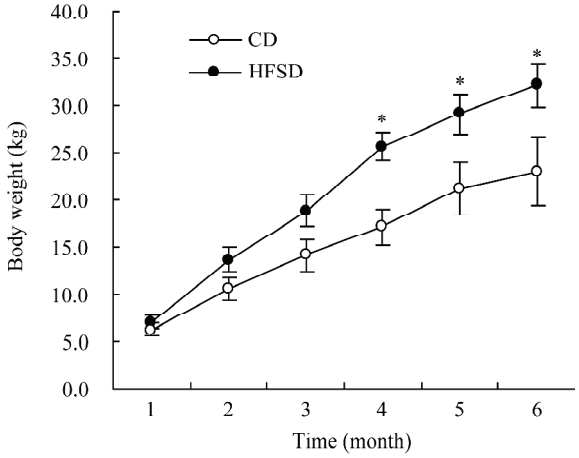

Fig. 2 Effects on body weight in swine fed a control diet (CD) or a high-fat/high-sucrose diet (HFSD)

HFSD feeding caused faster body growth in minipigs. The body weight gain was significantly different in HFSD-fed pigs than that in CD-fed pigs. Data were represented as mean±SD (n=5). *P<0.05 vs. CD.

plasma cholesterol throughout the experimental period. The fasting plasma triglycerides (TGs) in the HFSD group increased significantly with time, and the plasma free fatty acids (FFA) levels were also significantly higher in this group compared with the control group (**Table 1**).

Plasma adiponectin levels in swine

Serum adiponectin levels were determined by Western blot analysis with anti-gadiponectin antibody. All samples were tested under the same conditions. We took the same volume of blood as for SDS-PAGE, and incubated it with the same dilution of anti-adiponectin antibody and goat anti-rabbit IgG. A single injection of purified adiponectin led to a 2- to 3-fold elevation in circulating adiponectin levels (**Fig. 3**).

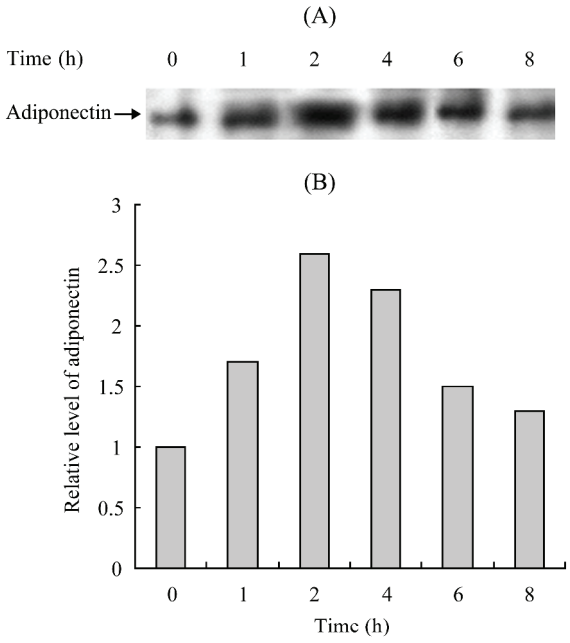

Fig. 3 Adiponectin levels in plasma samples from swine with induced diabetes

(A) Each lane shows the serum adiponectin level taken at 0, 1, 2, 4, 6 and 8 h after swine were treated with recombinant adiponectin as an intraperitoneal injection. (B) Densitometric analysis of the data of (A).

Effect of adiponectin on plasma glucose levels

Fasting plasma glucose levels were significantly high after HFSD feeding, up to 7.5 mM. To investigate the physiological role of recombinant human adiponectin, we injected recombinant adiponectin at 1 mg/kg body weight in the morning in a post-absorptive state. Serum glucose levels dropped significantly compared with the negative control in 4 h after injection (**Fig. 4**). We also measured glucose levels at 6 h and 8 h, but observed there were no significant differences between negative control and adiponectin-injected swine.

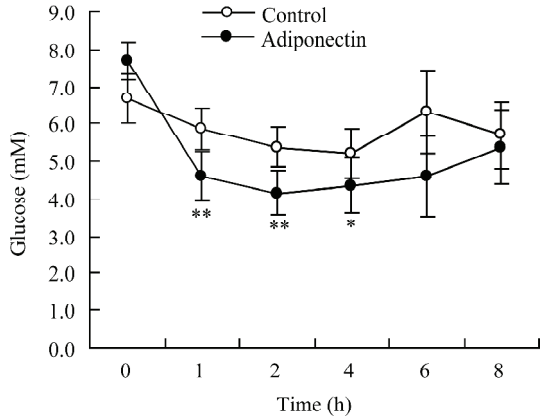

Fig. 4 Effects of adiponectin on plasma glucose levels in diabetic swine

Two groups of swine (n=3 per group) were treated with saline or adiponectin as described in "Materials and Methods". Blood samples were taken at 0, 1, 2, 4, 6, and 8 h after the test, and glucose concentrations were determined. Treatment with adiponectin significantly decreased glucose levels compared with the negative control at 1, 2 and 4 h. Data represent mean±SD. Statistical analysis was carried out with SPSS version 13.0 (SPSS, Chicago, USA). *P<0.05 and **P<0.01 vs. control.

Effect of adiponectin on glucose tolerance

In order to analyze the effects of treatment with adiponectin on glucose tolerance, we performed an OGTT test in 18 h-fasted swine from 2 groups. As shown in **Fig. 5**, the change in plasma glucose concentrations after the glucose load at control group exhibited marked glucose intolerance compared with those treated with adiponectin. The deficient glucose removal seen in the control group may have been caused by impairment of insulin sensitivity, because between the two groups, there is no difference in insulin secretion during OGTT (**Table 2**).

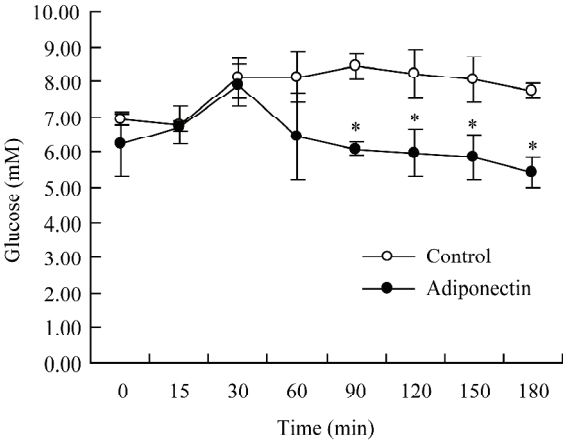

Fig. 5 Effects of adiponectin treatment on plasma glucose in an oral glucose tolerance test on diabetic swine

Two groups of swine (n=3 in each group) were treated with saline or adiponectin as described in "Materials and Methods". Blood samples were taken at 0, 15, 30, 60, 90, 120, 150 and 180 min after the test, and glucose concentrations were determined. Treatment with adiponectin significantly decreased the glucose levels compared with the negative control at 90, 120, 150 and 180 min. Data represent mean±SD. Statistical analysis was carried out with SPSS version 13.0 (SPSS). *P<0.05 vs. control.

Discussion

Adipose tissue not only stores excess energy, but also secretes a variety of proteins into circulating blood that influences systemic metabolism. These proteins include leptin [12], adiponectin [1–3,13], tumor necrosis factor (TNF)-α [14], plasminogen-activator inhibitor type 1 (PAI-1) [15], adipsin [16], and resistin [17], which are collectively known as adipocytokines [18]. Adipose tissue com-

Table 2 Oral glucose tolerance test results showing effects of adiponectin treatment on insulin secretion in swine

		0 min	30 min	60 min	90 min	120 min	150 min	180 min
Insulin (U/L)	Control	12.50±2.86	13.52±3.09	16.79±5.24	21.20±4.10	20.24±3.11	17.05±2.59	14.39±2.21
	Adiponectin	12.79±3.07	11.42±5.12	14.08±4.17	19.53±4.04	20.84±3.55	16.24±3.71	13.46±3.18

municates with other peripheral tissues by the release of these humoral mediators [19–21]. Thus, it is very interesting to investigate the dysregulated secretion of adipocytokines and their potential role in obesity-linked disorders, such as hypertension, atherosclerosis, insulin resistance, and type 2 diabetes.

Insulin resistance induced by a high-fat diet and associated with obesity is a major risk factor for diabetes, atherosclerosis, and cardiovascular diseases. In contrast to other adipocytokines, the plasma concentrations of adiponectin are not elevated but significantly reduced in obesity, insulin resistance, and coronary heart disease [5–7]. Researchers have found a correlation between insulin sensitivity and elevated adiponectin levels. For example, type 2 diabetic patients have lower adiponectin levels than nondiabetic controls, and chronic calorie restriction regimens lead to elevated adiponectin [22]. Furthermore, in rhesus monkeys predisposed to type 2 diabetes, the levels of circulating adiponectin decrease before the onset of hyperglycemia [5]. Because of adiponectin's beneficial effects on muscle and liver insulin sensitivity, its downregulation is suggested to contribute to the pathogenesis of these disorders. Adiponectin has been found to decrease the basal blood glucose level by increasing insulin sensitivity [23,24] and to increase the use of plasma free fatty acids through increasing expression of genes involved in fatty acid oxidation in mice [25].

The DNA sequences of human adiponectin are highly homologous with those of swine (86%). The amino acids sequence of human adiponectin is similar to that of the swine (83%). The high homology of the swine and human adiponectin suggests a more similar function of this protein in these two species compared with that in other mammalian species [26]. Swine have many characteristics similar to humans that raise the possibility that a swine model might be more appropriately extrapolated to human physiology. Swine are omnivores, easy to handle, raise few ethical considerations, offer similar size to adult humans, have several organ systems very similar to humans in terms of anatomy, physiology and metabolism, and test compounds can be given through all routes of delivery. Xi *et al*. [11] demonstrated that feeding HFSD to miniature pigs might induce altered glucose and lipid metabolism, as well as atherosclerotic fatty streaks. Therefore, we fed HFSD to a Chinese strain of miniature pigs to model human type 2 diabetes.

Although adiponectin functions in rodents such as mice and rats have been revealed, its effects in swine have not been reported. To promote the application of adiponectin into clinic, we conducted this experiment to measure the effect of short-term adiponectin on whole-body glucose metabolism and glucose tolerance. We injected the recombinant human adiponectin into experimental swine for the first time, with the result that glucose levels decreased but insulin secretion remained unchanged. Furthermore, it improved insulin sensitivity, tested by OGTT. Our results suggested that adiponectin treatment enhances animal models' insulin sensitivity and might serve as a therapy for human insulin resistance.

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