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Improvement of Insulin Resistance by Removal of Systemic Hydrogen Peroxide by PEGylated Catalase in Obese Mice

Mai Ikemura,[†] Makiya Nishikawa,*[,‡] Kenji Hyoudou,[†] Yuki Kobayashi,[†] Fumiyoshi Yamashita,[†] and Mitsuru Hashida[†,§]

Department of Drug Delivery Research and Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, and Institute for Integrated Cell-Material Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

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Abstract: Insulin resistance, a condition in which insulin action is impaired, is one of the characteristic features of type 2 diabetes. Excessive amounts of reactive oxygen species (ROS) interfere with the insulin signaling pathway, which leads to the progression of insulin resistance. To examine whether removal of systemic hydrogen peroxide is effective in improving insulin resistance, polyethylene glycol-conjugated catalase (PEG-catalase), a derivative with a long circulation half-life, was repeatedly injected into leptin-deficient *ob/ob* or high fat diet-induced obese mice for 16 or 10 consecutive weeks, respectively. Although *ob/ob* mice gradually gained weight with time irrespective of the treatment, repeated intraperitoneal injections of PEG-catalase significantly reduced glucose levels in the fed state. Glucose and insulin tolerance tests also showed PEG-catalase significantly improved glucose tolerance and insulin sensitivity in *ob/ob* mice, respectively. Similar but less marked results were obtained in the diet-induced obese mice. Treatment of 3T3-L1 adipocytes with glucose oxidase (GO) increased lipid hydroperoxide formation and reduced insulin-stimulated Akt phosphorylation. Addition of catalase or PEG-catalase significantly inhibited the GO-induced changes in adipocytes. These findings indicate that systemic removal of hydrogen peroxide by PEG-catalase activates the insulin signaling pathway and improves insulin resistance in obese mice.

Keywords: Insulin resistance; reactive oxygen species; hydrogen peroxide; catalase; *ob/ob* mice; high fat diet

Introduction

Insulin resistance, one of the characteristic features of type 2 diabetes, is the condition in which insulin action is impaired, leading to reduced glucose uptake in muscle cells and adipocytes, and to increased glucose production in hepatocytes. Insulin resistance is one of the major causes of the progression of hyperglycemia and type 2 diabetes.[1] Furthermore, it can also be an underlying cause of many serious diseases, including arteriosclerosis and hypertension.[2] These facts highlight the importance of the treatment of insulin resistance. Although some drugs have been developed to control insulin resistance, for example, thiazolidinediones (pioglitazone, rosiglitazone and troglitazone), there are safety

* Corresponding author. Mailing address: Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Shimoadachi-cho, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan. Tel: +81-75-753-4580. Fax: +81-75-753-4614. E-mail: makiya@pharm.kyoto-u.ac.jp.

† Department of Drug Delivery Research, Graduate School of Pharmaceutical Sciences.

‡ Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences.

§ Institute for Integrated Cell-Material Sciences.

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concerns about the clinical use of these drugs, including edema, weight gain and liver dysfunction.[3,4]

Oxidative stress induced by excessive production of reactive oxygen species (ROS) has been proposed to be involved in the pathogenesis of diabetes and its related syndromes.[5,6] Excessive amounts of ROS inhibit insulin signaling, kill pancreatic β-cells and impair the functions of some tissues and cells, for example, the kidney, retina and nerves.[7,8] Although some researchers have examined the effects of low molecular weight antioxidants on insulin resistance, the results were ambiguous.[9] Antioxidant enzymes are much more effective than low molecular weight antioxidants as far as the ability to scavenge ROS is concerned.[3,5,10] In addition, low molecular weight antioxidants have been shown to act as oxidants under some circumstances.[11] These considerations raise a possibility that antioxidant enzymes are more effective than low molecular weight antioxidants for the treatment of insulin resistance.

In prediabetic obesity, ROS are mainly generated in adipose tissues,[12] but they can be produced throughout the body when the condition becomes worse over time. The mechanisms of ROS production include[13] (1) metabolism of glucose and free fatty acids in mitochondria in various cells including muscle cells and adipocytes, (2) signaling via TNF-α in various types of cells, (3) increased NAD(P)H oxidase activity in phagocytic and nonphagocytic cells, and (4) the interaction of advanced glycation end-products with their cell-surface receptors. Therefore, continuous removal of systemic ROS could be effective in inhibiting insulin resistance.

Hydrogen peroxide, an ROS, has a long half-life and easily diffuses across the plasma membrane.[14,15] Within cells, hydrogen peroxide activates transcription factors, including nuclear factor-κB (NF-κB), and a variety of genes, including ones involved in the progression of insulin resistance, are upregulated.[7] In addition, it has been reported that hydrogen peroxide inhibits intracellular insulin signaling.[16,17] In a mouse model of obesity, increased levels of hydrogen peroxide were reported in plasma and white adipose tissue.[12] These pieces of experimental evidence suggest that hydrogen peroxide plays an important role in the progression of insulin resistance compared with other ROS.

Based on these considerations, catalase, an enzyme detoxifying hydrogen peroxide, was selected as a compound to improve insulin resistance. We have shown that the therapeutic effects of catalase are greatly increased by optimizing the tissue distribution properties by chemical modification.[18−22] In the present study, to increase the circulation time of

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catalase after administration, a catalase derivative conjugated with polyethylene glycol (PEG-catalase) was selected for the improvement of insulin resistance. PEG-catalase was repeatedly injected into two types of *in vivo* models of insulin resistance, i.e., *ob/ob* and high fat diet (HFD)-induced obese mice, and body weight and blood glucose level were measured to examine whether continuous removal of hydrogen peroxide is effective in improving insulin resistance. The effect of catalase on insulin signaling was also examined by measuring the phosphorylation of Akt in adipocytes.

Experimental Section

Chemicals. Catalase (bovine liver), insulin and glucose oxidase (GO) were obtained from Sigma Chemical (St. Louis, MO, USA). Bovine serum (BS) and penicillin−streptomycin glutamine (PSG) were obtained from Invitrogen Corporation (Grand Island, NY, USA). Dulbecco's modified Eagle's minimum essential medium (DMEM) was obtained from Nissui Pharmaceutical (Tokyo, Japan).

Animals. C57BL/6 mice and *ob/ob* mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Animals were maintained under conventional housing conditions, and all animal experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocols for animal experiments were approved by the Animal Experimentation Committee of Graduate School of Pharmaceutical Sciences of Kyoto University.

Synthesis of PEG-catalase. According to the published method,[23] PEG-catalase was synthesized by conjugating catalase with 2,4-bis(*O*-methoxypolyethylene glycol)-6-chloro-*s*-triazine (average molecular weight of 10,000; Seikagaku Corporation, Japan), which was selected based on the remaining enzymatic activity (unpublished data). The molecular weights of catalase and PEG-catalase were 240,000 and 840,000, respectively, and about 24% of amino groups of catalase were estimated to be modified with PEG in PEG-catalase.[23] The remaining activity of PEG-catalase was 96% of unmodified catalase.[23]

Administration of PEG-catalase to *ob/ob* Mice. Five-week-old male C57BL/6 mice and *ob/ob* mice were used as nondiabetic (lean) and insulin-resistant mice, respectively. Mice were fed with normal fat diet (NFD) (MF, 3.60 kcal/g, 13 kcal % fat; Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. A group of *ob/ob* mice received intraperitoneal injections of a saline solution of PEG-catalase three times a week at a dose of 5000 catalase units/day on Monday and Wednesday and of 7500 catalase units/day on Friday. Another group of *ob/ob* mice and a group of lean mice received the same volume of saline solution according to the same protocol. The treatment continued for 16 weeks and the body weight of the mice was recorded weekly.

Measurement of Glucose Levels in the Fed State. Glucose levels in the fed state were measured in the morning with *ad libitum* feeding from 5 to 16 weeks after the onset of treatment in *ob/ob* mice. Blood samples were obtained from the tail vein, and blood glucose levels were determined with an ACCU-CHEK Active meter (Sanko Junyaku Co., Ltd., Japan).

Glucose Tolerance Test. A glucose tolerance test was performed after a 12 week treatment with PEG-catalase as described above. After fasting for 4 h, mice were injected intraperitoneally with D-(+)-glucose (Wako Pure Chemical Industries, Ltd., Japan) at a dose of 1.5 g/kg body weight. Blood samples were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min after glucose injection, and blood glucose levels were measured with an ACCU-CHEK Active meter. When the level was over 600 mg/dL, a G-checker (Sanko Junyaku Co., Ltd., Japan) was used for the measurement.

Insulin Tolerance Test. An insulin tolerance test was performed after a 16 week treatment with PEG-catalase as described above. After fasting for 4 h, mice were injected subcutaneously with insulin at a dose of 0.1 units/kg body weight. Blood samples were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min after insulin injection, and blood glucose levels were measured as described above.

High Fat Diet-Induced Obese Mice. Three-week-old male C57BL/6 mice fed with NFD or HFD (HFD60, 5.06 kcal/g, 62 kcal % fat; Oriental Yeast Co., Tokyo, Japan) were used as nondiabetic and insulin-resistant mice, respectively. Mice were given each diet and water *ad libitum*. Dosage regimen was the same as that of the first experiment, except for the difference in the duration of the treatment (10 weeks). Glucose levels in the fed state were measured at random times from 5 to 10 weeks after the onset of treatment with PEG-catalase, and glucose tolerance tests were performed after 7 week treatment as described above.

Cell Culture and Treatment. 3T3-L1 preadipocytes (DS Pharma Biomedical Co., Ltd., Osaka, Japan) were cultured in a DMEM containing 10% BS and PSG in 12-well plates. When cells reached confluence, the culture medium was replaced with a DMEM containing 10% fetal bovine serum (FBS, Equitech-bio, Inc., Kerrville, TX, USA), PSG, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (Nacalai Tesque, Ink., Kyoto, Japan), 10 μM dexamethasone (Nacalai Tesque, Ink.) and 10 μg/mL insulin, and cells were incubated for 48 h. Then, cells were incubated for an additional 48 h in the same medium without IBMX and dexamethasone for differentiation to adipocytes. After full differentiation, cells were mixed with GO (0.004 units/mL) in combination with or without a catalase derivative (catalase or PEG-catalase, 500 units/mL). This treatment was repeated every 48 h. Then, 5 days after the onset of treatment, cells were harvested for the following measurements.

Measurement of Lipid Hydroperoxide. The amount of lipid hydroperoxide in 3T3-L1 adipocytes was measured

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using a Spy-LHP fluorescent probe (Dojindo, Kumamoto, Japan) as reported previously.[24] Fluorescent intensity was measured using a fluorescent activated cell sorter (Becton Dickinson, Franklin Lakes, NJ, USA).

Western Blot Analysis. 3T3-L1 adipocytes were treated with 100 nM insulin for 15 min. Cells were lysed with RIPA lysis buffer (Santa Cruz Biotechnology, Inc., CA, USA) and the lysates were centrifuged at 10000*g* for 10 min at 4 °C. The supernatant was diluted in a sample buffer (0.25 mM Tris-HCl (pH 6.8), 10% 2-mercaptoethanol, 4% SDS, 10% sucrose, and 0.004% bromphenol blue). Proteins were separated on SDS−PAGE, and transferred onto PVDF membranes (Hybond-P, Amersham Biosciences, Buckinghamshire, U.K.), which were then blocked with 0.4% BSA in TBS (250 mM Tris and 1.5 M NaCl, pH 7.4) containing 0.1% Tween20 (TBS-T) for 1 h. The membrane was probed with anti-Akt or anti-phospho-Akt (Ser473) antibody (Cell Signaling Technology, Inc.), incubated overnight in TBS-T containing 0.4% BSA, washed three times with TBS-T, and incubated for 1 h at room temperature with horseradish peroxidase-conjugated sheep anti-rabbit immunoglobulin (Amersham Biosciences, Buckinghamshire, U.K.) in TBS-T. Protein bands were visualized using Immobilon Western Chemiluminescent HRP Substrate (Millipore Corporation, Billerica, USA) and the luminescence was detected using a cooled CCD camera (LAS-3000, Fujifilm, Tokyo, Japan) according to the manufacturer's instructions.

Statistical Analysis. Differences were statistically evaluated by one-way ANOVA followed by the Student−Newman−Keuls multiple comparison test. The significance of differences was set at $P < 0.05$.

Results

Changes in Body Weight and Blood Glucose Level Following Repeated Injections of PEG-catalase. The body weight of *ob/ob* and HFD-fed mice increased with time, and the increase was much greater than that of the lean and NFD-fed mice, respectively (Figure 1). The repeated injections of PEG-catalase did not affect the body weight of *ob/ob* and HFD-fed mice. In the state of insulin resistance, the fasting glucose level is not so high, but it takes more time to return to the normal level when the blood glucose level increases after a meal. To examine the effect of PEG-catalase on the blood glucose level, blood was sampled from mice in the fed state at several points from 5 weeks of treatment to the end of the experiments. The blood glucose level of the saline-treated *ob/ob* and HFD-fed mice was significantly higher than that of the lean and NFD-fed mice, respectively (Figure 2). This high blood glucose level of both obese mice could be explained by assuming that the mice had developed insulin resistance. The PEG-catalase-treated obese mice showed a significantly lower blood glucose level than the saline-treated ones, suggesting that the progression of insulin resistance was partly prevented by repeated injections of PEG-catalase.

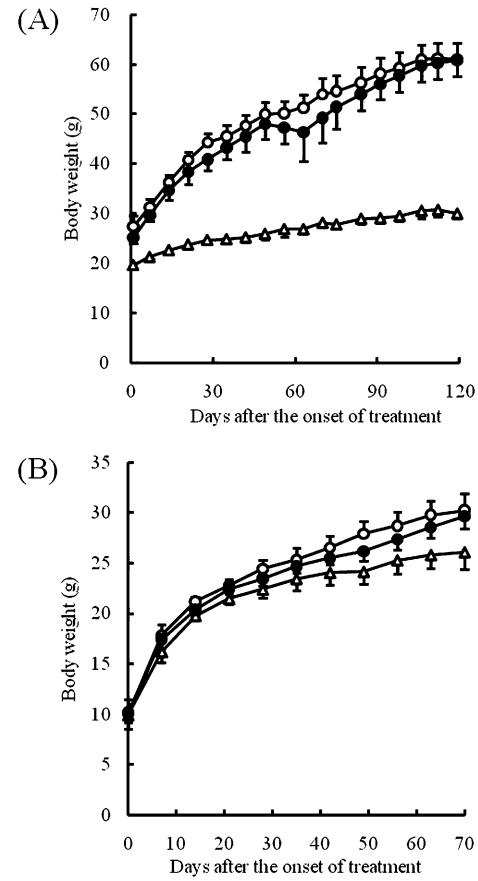

Figure 1. Body weight change in *ob/ob* and HFD-fed mice. (A) Body weight changes of the lean (white triangle), saline-treated *ob/ob* mice (white circle) and PEG-catalase-treated *ob/ob* mice (black circle). (B) Body weight changes of NFD-fed mice (white triangle), saline-treated HFD-fed mice (white circle) and PEG-catalase-treated HFD-fed mice (black circle). Results are expressed as mean ± SD ($n = 4-5$).

Effect of Repeated Injections of PEG-catalase on Insulin Resistance: Glucose and Insulin Tolerance Tests. To confirm the effect of PEG-catalase on insulin resistance, a glucose tolerance test was performed (Figure 3). The fasting glucose level of the *ob/ob* mice (at 0 min in the figure) was significant higher than that of the lean mice (Figure 3A). A low fasting glucose level was observed in the PEG-catalase-treated *ob/ob* mice. Injection of glucose rapidly increased the blood glucose level in all groups tested. In the lean mice, the increase was not so marked compared with that in the *ob/ob* mice, and the level returned to the initial level within 120 min. The *ob/ob* mice showed a greater increase in the blood glucose level, irrespective of the treatment. However, the increase declined rapidly in the PEG-catalase-treated mice. HFD-fed mice also showed higher glucose levels than NFD-fed mice during the test (Figure 3B). A rapid decline in the glucose level was observed in the PEG-catalase-treated mice.

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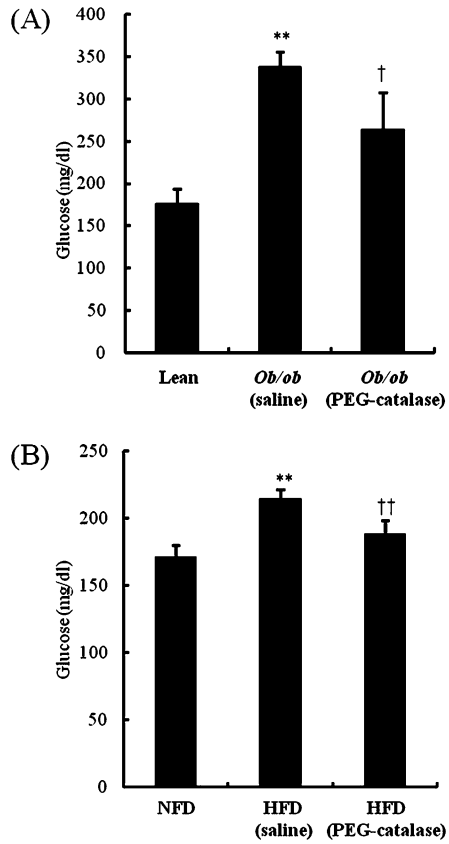

Figure 2. Glucose levels in the fed state of (A) *ob/ob* and (B) HFD-fed mice. Blood glucose levels were measured at random times with *ad libitum* feeding from 5 weeks after the start of treatment to the end of each experiment. Results are expressed as mean ± SD (n = 4−5). $^{**}P < 0.01$, statistically significant difference compared with the lean or NFD-fed mice. $^{\dagger}P < 0.05$, statistically significant difference compared with the *ob/ob* (saline) mice. $^{\dagger\dagger}P < 0.01$, statistically significant difference compared with HFD-fed mice (saline).

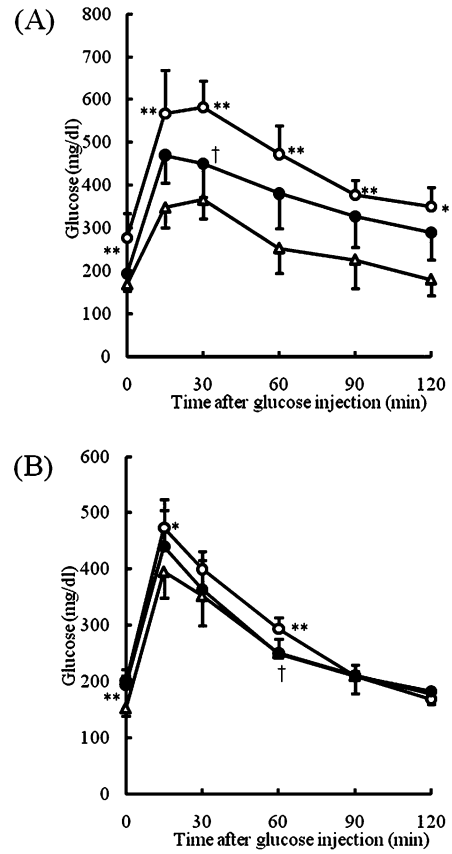

Figure 3. Effect of PEG-catalase on glucose tolerance. (A) Blood glucose levels during the glucose tolerance test performed using the lean (white triangle), saline-treated *ob/ob* mice (white circle) and PEG-catalase-treated *ob/ob* mice (black circle) that had received a 12 week treatment. (B) Blood glucose levels during the glucose tolerance test performed using the NFD-fed mice (white triangle), saline-treated HFD-fed mice (white circle) and PEG-catalase-treated HFD-fed mice (black circle) that had received a 7 week treatment. Results are expressed as mean ± SD (n = 4−5). $^{*}P < 0.05$, $^{**}P < 0.01$, statistically significant difference compared with the lean or NFD-fed mice. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, statistically significant difference compared with the *ob/ob* (saline) or saline-treated HFD-fed mice.

In an insulin tolerance test, the blood glucose level decreased very quickly in the lean mice just after insulin injection (Figure 4). In contrast, the level of the saline-treated *ob/ob* mice did not show any response at all to the insulin injection. However, the blood glucose level of the PEG-catalase-treated *ob/ob* mice decreased following the injection, indicating that the insulin sensitivity of *ob/ob* mice is improved by repeated injections of PEG-catalase.

Effect of Catalase Derivatives on GO-Induced Oxidative Stress in Adipocytes. The results obtained suggest that continuous removal of hydrogen peroxide is effective in preventing the development of insulin resistance in *ob/ob* mice. To examine how hydrogen peroxide affects insulin signaling in adipocytes, 3T3-L1 adipocytes were treated with GO, which continuously generates hydrogen peroxide in the presence of glucose. A high level of lipid hydroperoxide was detected in the GO-treated cells compared with that in the untreated cells (Figure 5), indicating that the GO treatment induced oxidative stress in adipocytes. This GO-induced increase in the level of lipid hydroperoxide was significantly inhibited by addition of catalase or PEG-catalase.

Effect of Catalase Derivatives on Insulin Signaling in Adipocytes. Insulin-stimulated phosphorylation of Akt was investigated in GO-treated adipocytes by Western blotting. The level of Akt phosphorylation was normalized to the total amount of Akt. The level of Akt phosphorylation in the GO-treated adipocytes was decreased to 30% of the control group (NT) (Figure 6). This phosphorylation was almost completely restored in the catalase- (98%) or PEG-catalase treated cells (110%). Because Akt phosphorylation is required for insulin signaling in adipocytes and muscle cells, these results suggest that Akt phosphorylation in adipocytes is involved in the action of PEG-catalase to improve insulin resistance in *ob/ob* mice.

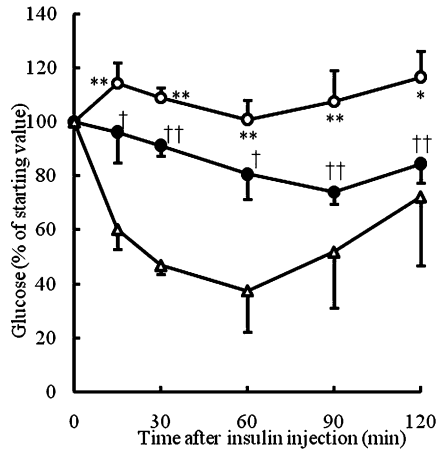

Figure 4. Effect of PEG-catalase on insulin sensitivity. Blood glucose levels during the insulin tolerance test performed using the *ob/ob* mice that had received a 16 week treatment. Results indicate the time course of the % of starting glucose levels after insulin injection. Results are expressed as mean ± SD ($n = 4-5$). $^{*}P < 0.05$, $^{**}P < 0.01$, statistically significant difference compared with the lean mice. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, statistically significant difference compared with the *ob/ob* (saline).

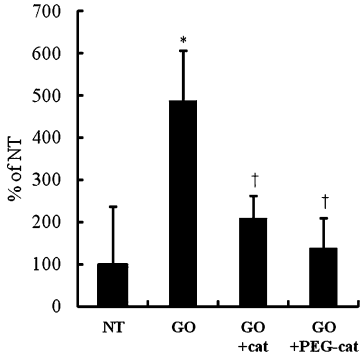

Figure 5. Effect of catalase derivatives on lipid hydroperoxide levels in GO-treated cells. 3T3-L1 adipocytes were treated with GO, and catalase (cat) or PEG-catalase (PEG-cat). At 24 h after the last treatment, lipid hydroperoxide levels were evaluated using a fluorescent probe, Spy-LHP. Results are expressed as mean ± SD ($n = 3$). $^{*}P < 0.05$, statistically significant difference compared with no treated cells. $^{\dagger}P < 0.05$, statistically significant difference compared with GO-treated cells.

Discussion

The phosphorylation of Akt by insulin has been frequently used as a marker of insulin signaling, because Akt is downstream of insulin receptors in adipocytes and Akt phosphorylation leads to increased glucose uptake via GLUT4.[13,25] Previous studies showed that oxidative stress inhibits insulin-induced Akt phosphorylation,[16,26] although others reported contradictory results.[27,28] We clearly demonstrated that the insulin-induced Akt phosphorylation in adipocytes was significantly impaired by the addition of GO, and this was effectively prevented by catalase or PEG-catalase (Figure 6). Because the treatment significantly reduced the level of oxidative stress in adipocytes, these results strongly suggest that catalase or PEG-catalase can effectively restore insulin-induced Akt phosphorylation by detoxifying hydrogen peroxide.

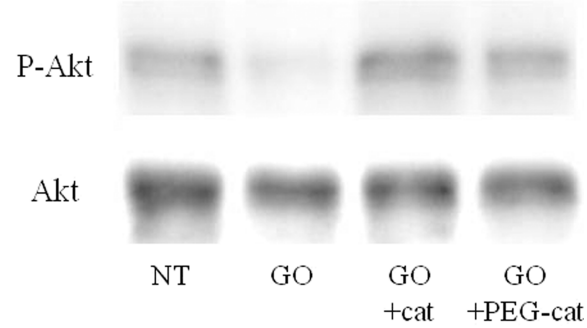

Figure 6. Effect of catalase derivatives on insulin-stimulated Akt phosphorylation in GO-treated cells. 3T3-L1 adipocytes were treated with GO, and catalase or PEG-catalase. At 24 h after the last treatment, cells were stimulated with insulin and intracellular fractions were collected. Immunoblots show phospho-Akt (P-Akt) (upper) and Akt (lower) levels on acute stimulation of cells with insulin. Equivalent amounts of protein were added to each lane. Blots shown are representive of 3 independent experiments.

Because of impaired insulin signaling, it takes a long time for the increased blood glucose level to fall under insulin-resistant conditions. The *ob/ob* and HFD-fed mice showed a significantly higher glucose level in the fed state compared with the lean and NFD-fed mice, respectively (Figure 2). These results indicate that the glucose level in the fed state can be a good parameter to evaluate insulin resistance as reported previously.[9] The blood glucose level in the fed state was significantly lower in the PEG-catalase-treated obese mice than that in the saline-treated obese mice, indicating that PEG-catalase accelerates the reduction in blood glucose. This notion is supported by the results of the glucose tolerance test (Figure 3), which is a widely used test to evaluate insulin resistance; the increased blood glucose level produced by glucose injection fell more quickly in the PEG-

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catalase-treated *ob/ob* and HFD-fed mice. In both obese mice, PEG-catalase improved insulin resistance, including glucose levels in the fed state and glucose tolerance. However, the degree of improvement was less pronounced in the HFD-fed mice than in *ob/ob* mice. This could be explained by the fact that the degree of obesity and insulin resistance of the HFD-fed mice used in the present study was less than those of the *ob/ob* mice. Although the fasting glucose levels of *ob/ob* mice were somewhat, but not significantly, reduced by PEG-catalase (Figure 3A), those of HFD-fed mice were not affected by the treatment. These results suggest that PEG-catalase has no significant effect to reduce the fasting glucose level in obese mice.

The sensitivity to insulin was separately evaluated by the insulin tolerance test. The PEG-catalase-treated *ob/ob* mice showed a slight reduction in blood glucose level after administration of insulin (Figure 4), which was not observed at all in the saline-treated group. These results are direct evidence that the sensitivity to insulin of *ob/ob* mice is increased by PEG-catalase. Thus, it can be speculated that, in the PEG-catalase-treated *ob/ob* mice, an increase in the blood glucose level leads to insulin secretion from pancreatic β cells and then the insulin secreted reduces the blood glucose level through binding to its receptors on the surface of muscle cells and adipocytes, followed by activation of insulin signaling.

The saline-treated *ob/ob* mice did not respond to insulin, suggesting that, even if a high blood glucose level triggers insulin secretion, the blood glucose level is not affected by the secretion because of the impaired insulin signaling pathway. In turn, the restored insulin sensitivity in the PEG-catalase-treated mice suggests that continuous removal of systemic hydrogen peroxide is an effective approach to restoring the insulin signaling pathway, as observed in cultured adipocytes. In many cases, insulin resistance is induced by obesity, which is characterized by adipocyte hypertrophy.[29] Accompanied by adipocyte hypertrophy, generation of adiponectin from adipose tissue is reduced, and that of TNF-α and resistin is increased, causing insulin resistance.[29,30] If larger adipocytes are converted to smaller ones, insulin resistance is thought to be improved. The finding that the volume of adipose tissue is closely related to the body weight[31] suggests that the loss of body weight increases insulin sensitivity. In the present study, the body weight of *ob/ob* and HFD-fed mice was hardly affected by the repeated injections of PEG-catalase three times a week for 16 and 10 weeks, respectively (Figure 1). These results are in good agreement with other reports regarding the effects of antioxidants on insulin resistance in mice.[9,12] Therefore, it can be concluded that repeated injection of PEG-catalase improves insulin resistance not by reduction of body weight but by removing systemic hydrogen peroxide.

A variety of PEG molecules, which are diverse in size and shape, are available for protein modification. In general, branched and longer PEG chain extends the elimination half-life of proteins but reduces their biological activity *in vitro*, so the balance of these two is important for the development of PEG-modified proteins.[32,33] We selected 2,4-bis(*O*-methoxypolyethylene glycol)-6-chloro-*s*-triazine with molecular weight of 10,000, based on remaining enzymatic activity (unpublished data). PEG modification of catalase significantly increased the plasma half-life of catalase from 0.3 to 14 h.[21] This increase was associated with increased therapeutic activity of PEG-catalase in a variety of disease models.[20,21,34] In the present study, therefore, we used PEG-catalase, not catalase, to remove hydrogen peroxide in mice.

The large molecular weight of PEG-catalase as well as the shield of PEG chains could prevent PEG-catalase from being taken up by cells. This speculation may raise a concern about the effect of PEG-catalase on insulin resistance, because ROS interfere with the insulin signaling pathway within cells. However, hydrogen peroxide, the ROS that catalase scavenges, is a neutral small molecule and easily diffuses across the plasma membrane.[14,15] Therefore, although PEG-catalase has hardly any access to the inside of cells, the reduction in the extracellular level of hydrogen peroxide by PEG-catalase will lead to a reduction in its intracellular level.

The dosing regimens of PEG-catalase require improvements, when used for patients with insulin resistance. Combining PEG-catalase with controlled-released formulations is one solution to reduce the frequency of administration. Previous studies have demonstrated that the sustained release of PEG-catalase or cationized catalase from gelatin hydrogel formulations is effective in inhibiting tumor metastasis compared with its injection as a solution.[35,36]

In conclusion, PEG-catalase effectively improved insulin resistance by scavenging systemic hydrogen peroxide. Insulin

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sensitivity and glucose tolerance were improved by repeated injections of PEG-catalase. It was also found that this inhibitory effect involves the restoration of insulin signal that is impaired by GO-induced hydrogen peroxide. These findings indicate that the retention of catalase in the blood circulation by repeated injections of PEG-catalase is an effective approach to improving the progression of insulin resistance.

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