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(54) **INSULIN RESISTANCE IMPROVING AGENTS**

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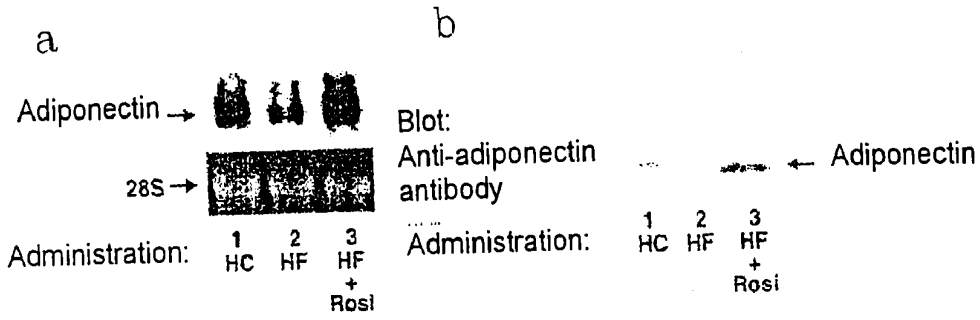
(57) **ABSTRACT**

The invention provides an insulin resistance improving agent and a therapeutic agent for type 2 diabetes, which contain a C-terminal globular domain of adiponectin, adiponectin, or a gene for the domain or adiponectin. The invention also provides a method for improving insulin resistance and treating type 2 diabetes by administering the aforementioned agent (s).

Fig. 1


a
Adiponectin
28S
Administration:
1 HC
2 HF
3 HF + Rosi
b
Blot:
Anti-adiponectin antibody
Adiponectin
Administration:
1 HC
2 HF
3 HF + Rosi

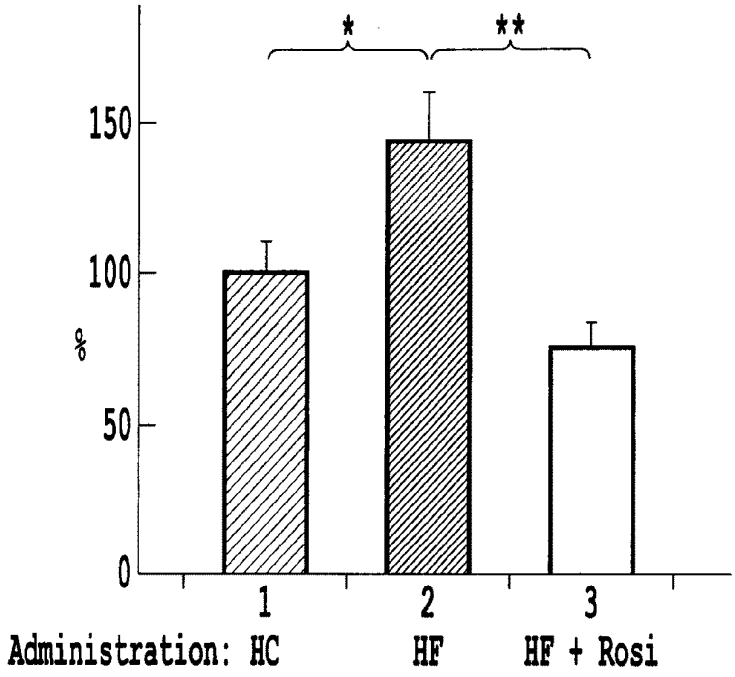

*
**
150
100
%
50
0
1
2
3
Administration: HC
HF
HF + Rosi

Fig. 2A

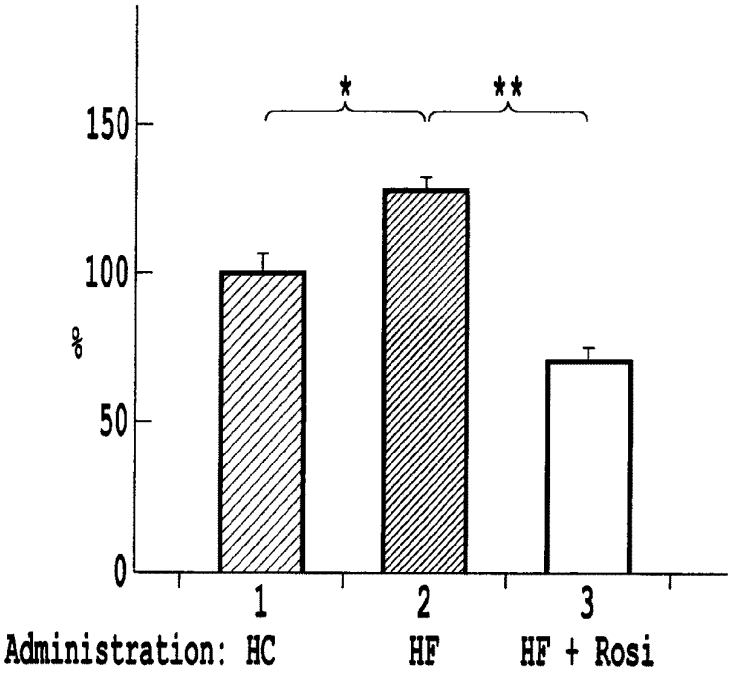

*
**
150
100
%
50
0
1
2
3
Administration: HC
HF
HF + Rosi

Fig. 2B

Fig. 3

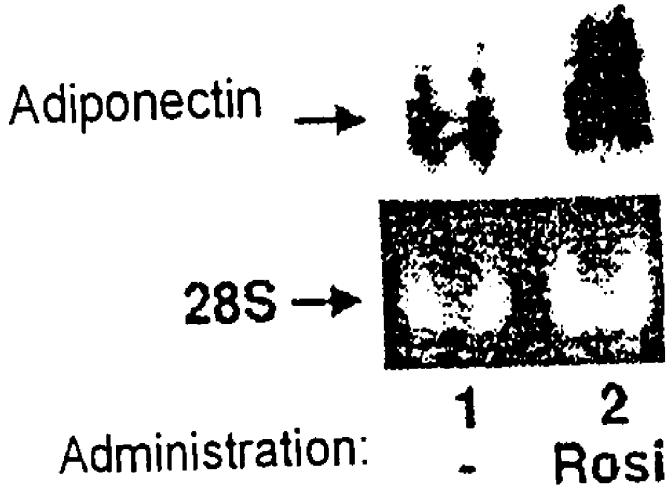


Fig. 4

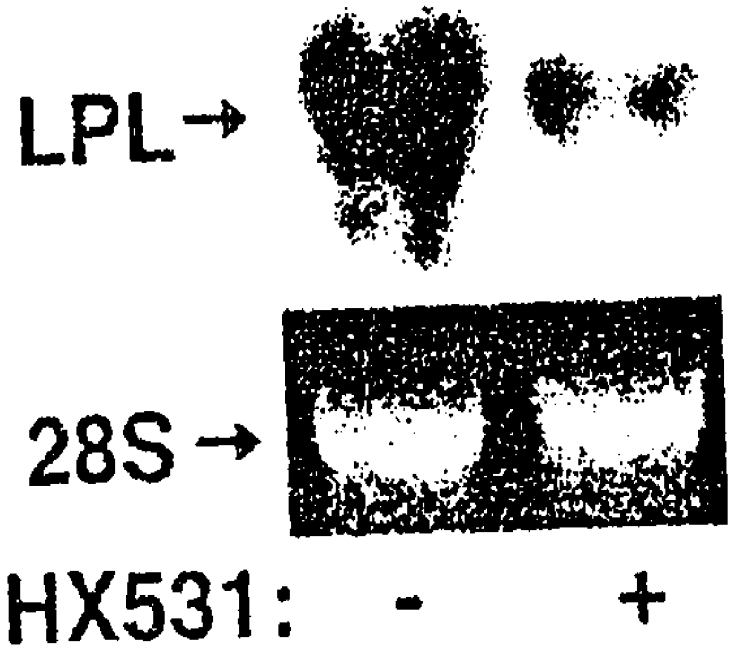


Fig. 5

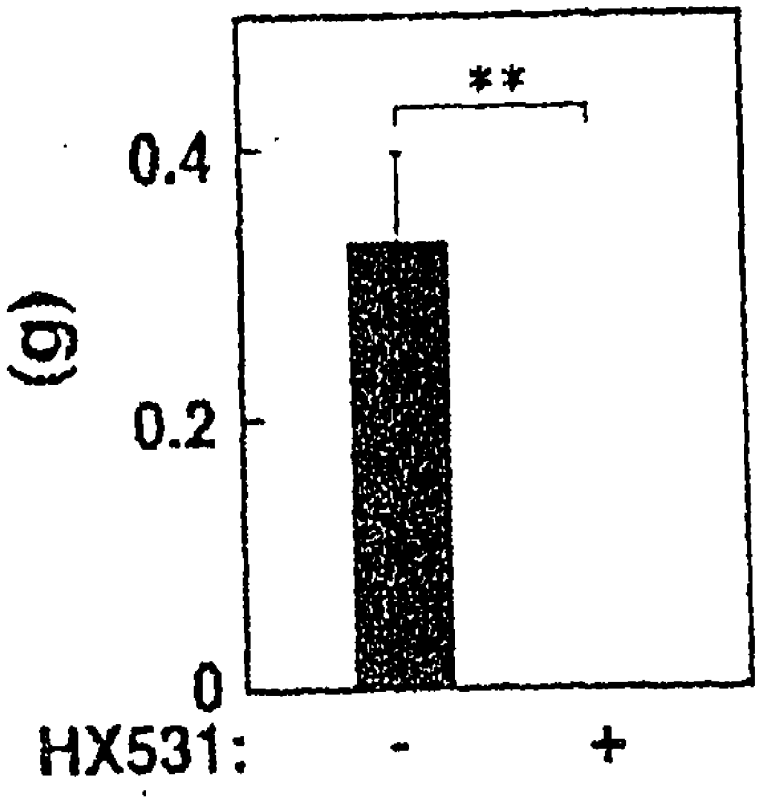
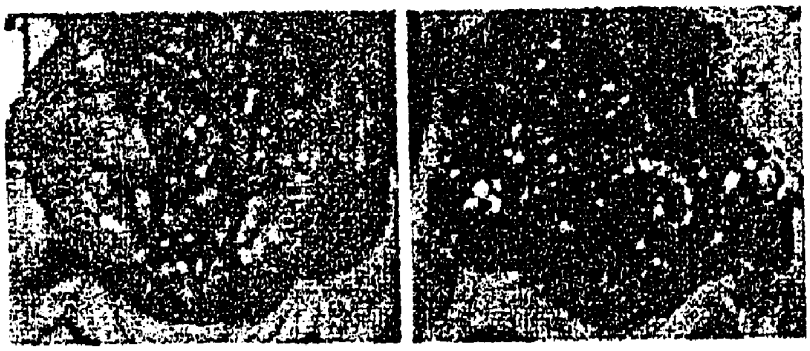


Fig. 6



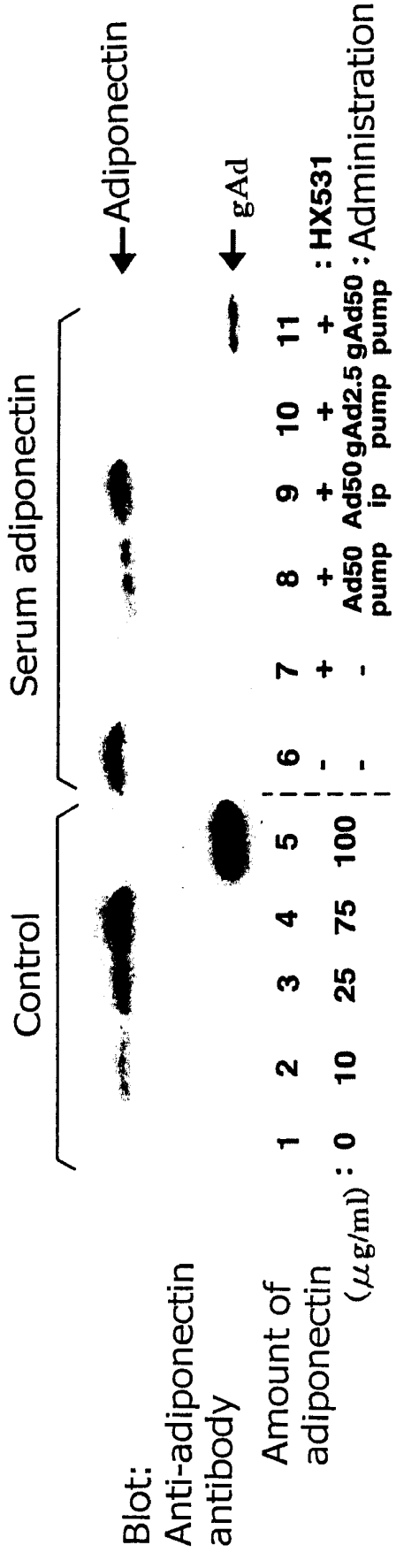

Blot:
Anti-adiponectin antibody
Control
Serum adiponectin
Adiponectin
gAd
Amount of adiponectin (μg/ml) : 0 10 25 75 100 - -
1 2 3 4 5 6 7 8 9 10 11
- - + + + + + + + + +
Ad50 pump
Ad50 ip
gAd2.5 pump
gAd50 pump
: HX531
: Administration

FIG. 7

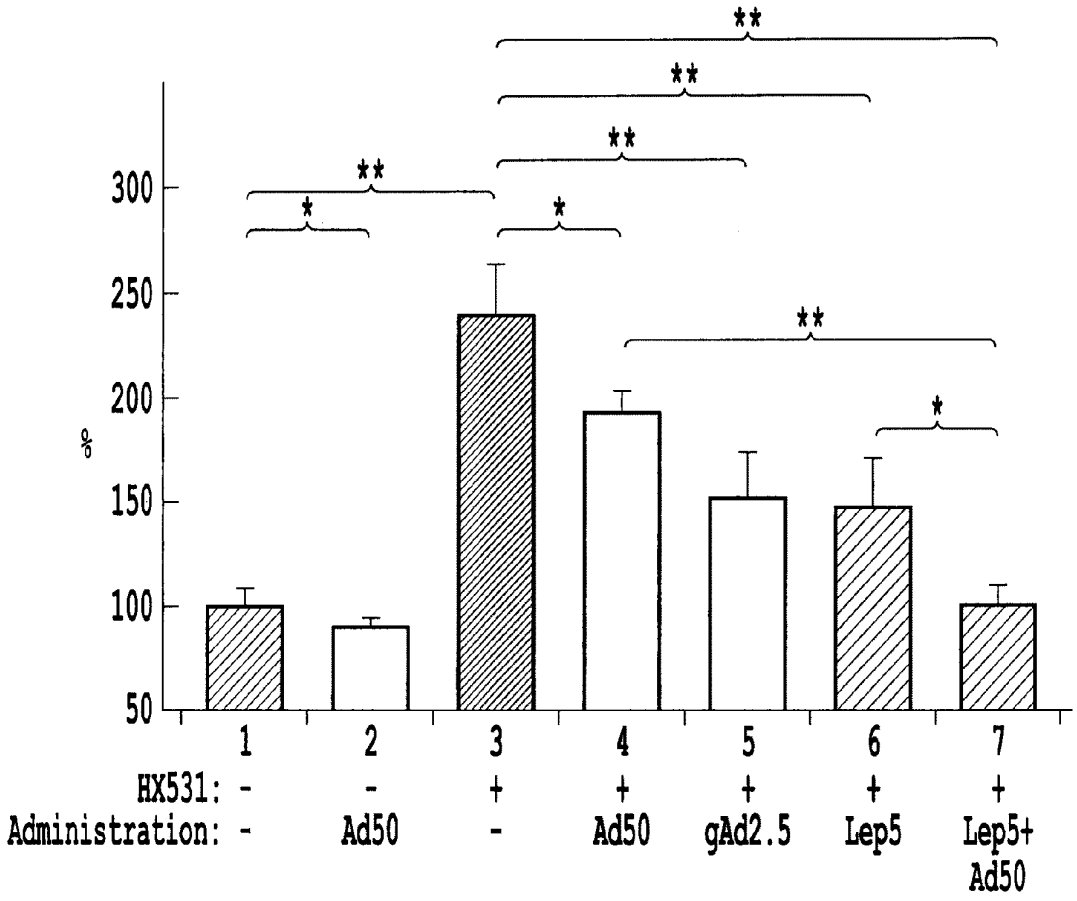

**
**
**
**
*
*
**
*
300
250
200
150
100
50
%
1
2
3
4
5
6
7
HX531: -
-
+
+
+
+
+
Administration: -
Ad50
-
Ad50
gAd2.5
Lep5
Lep5+
Ad50

Fig. 8

Fig. 9

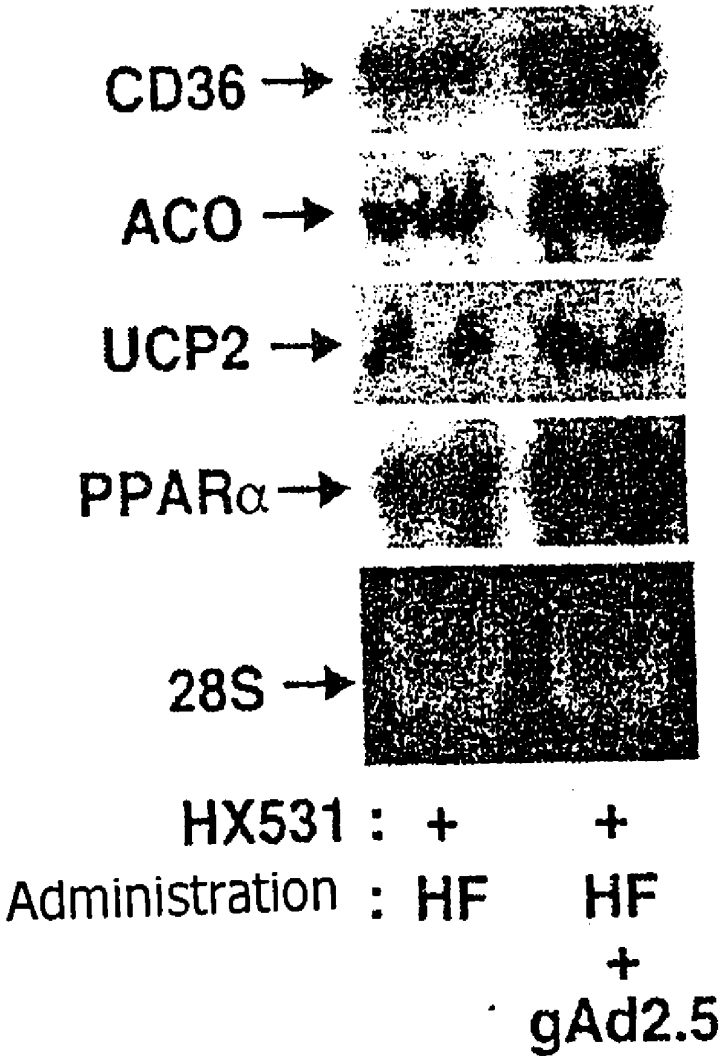


Fig. 10

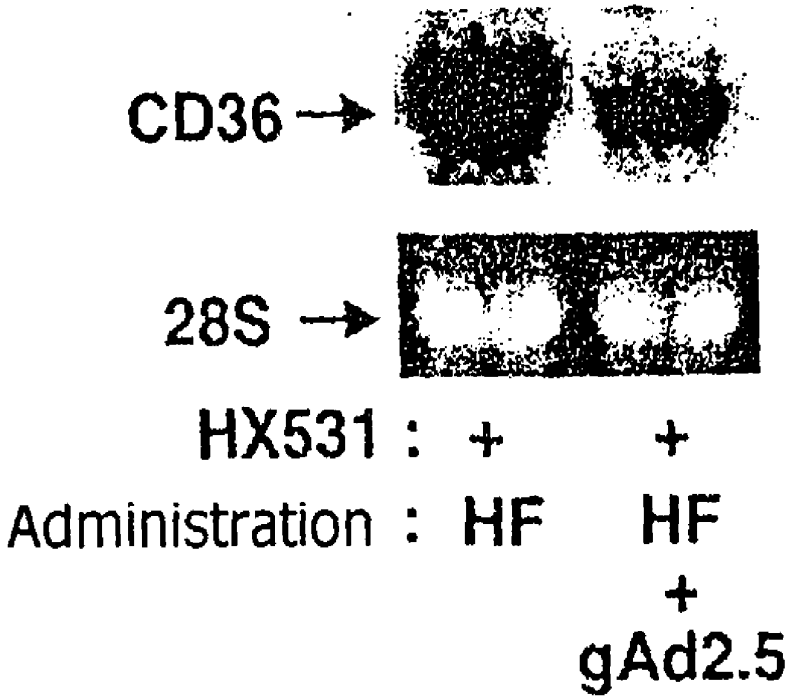


Fig. 11

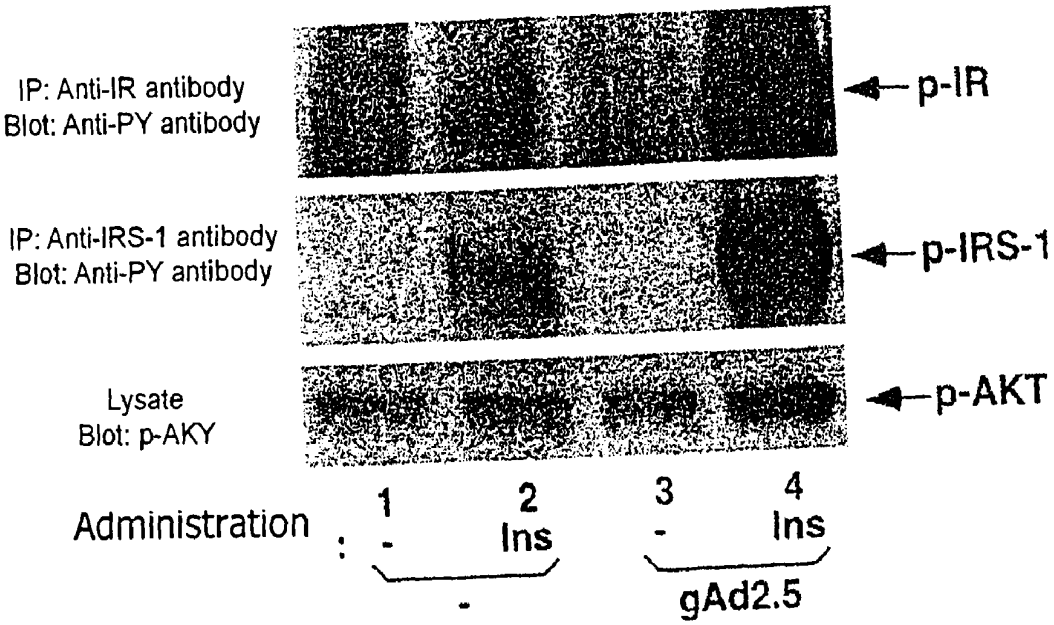

IP: Anti-IR antibody
Blot: Anti-PY antibody
p-IR
IP: Anti-IRS-1 antibody
Blot: Anti-PY antibody
p-IRS-1
Lysate
Blot: p-AKY
p-AKT
Administration :
1
-
2
Ins
3
-
4
Ins
-
gAd2.5

Fig. 12

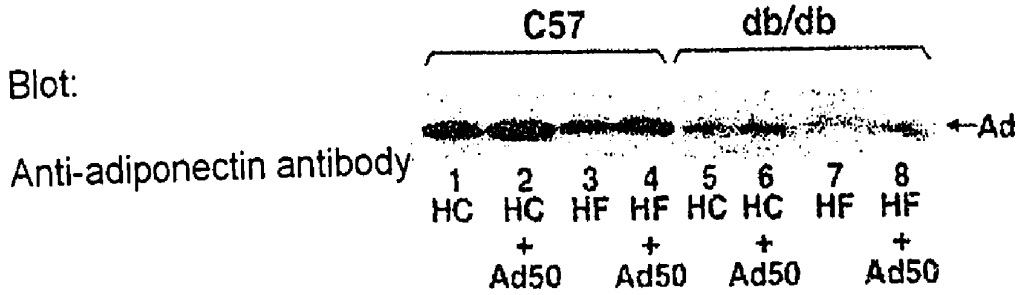

C57
db/db
Blot:
Anti-adiponectin antibody
←Ad
1 HC
2 HC + Ad50
3 HF
4 HF + Ad50
5 HC
6 HC + Ad50
7 HF
8 HF + Ad50

Fig. 13

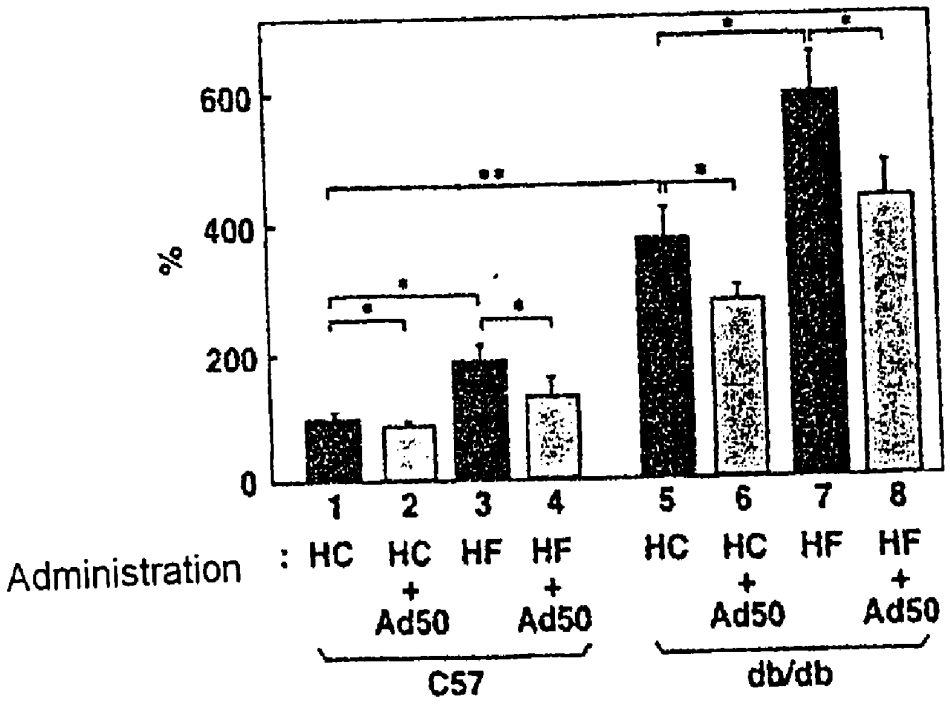

600
400
200
0
%
1
2
3
4
5
6
7
8
Administration : HC
HC + Ad50
HF
HF + Ad50
HC
HC + Ad50
HF
HF + Ad50
C57
db/db

Fig. 14

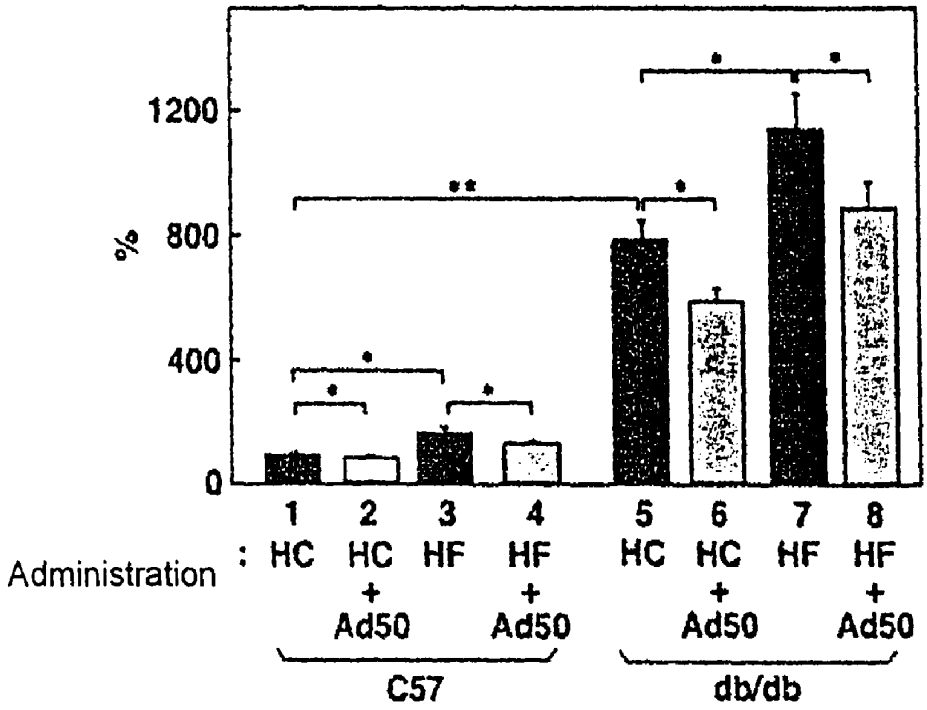

1200
%
800
400
0
1 2 3 4 5 6 7 8
Administration : HC HC + Ad50 HF HF + Ad50 HC HC + Ad50 HF HF + Ad50
C57
db/db

Fig. 15

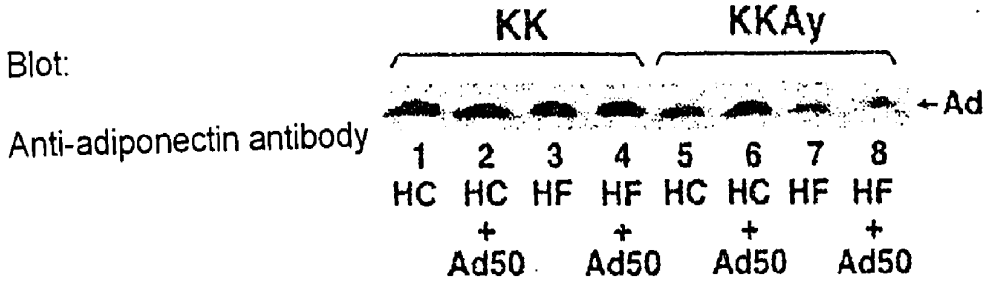

KK
KKAy
Blot:
Anti-adiponectin antibody
←Ad
1 2 3 4 5 6 7 8
HC HC HF HF HC HC HF HF
+ + + +
Ad50 Ad50 Ad50 Ad50

Fig. 16

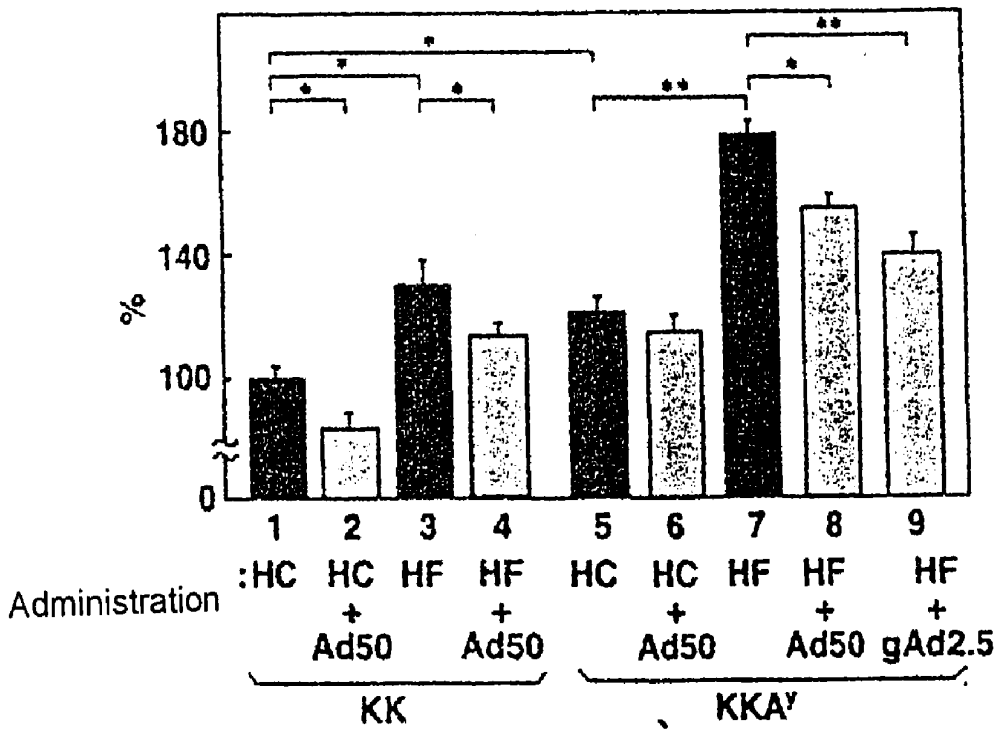
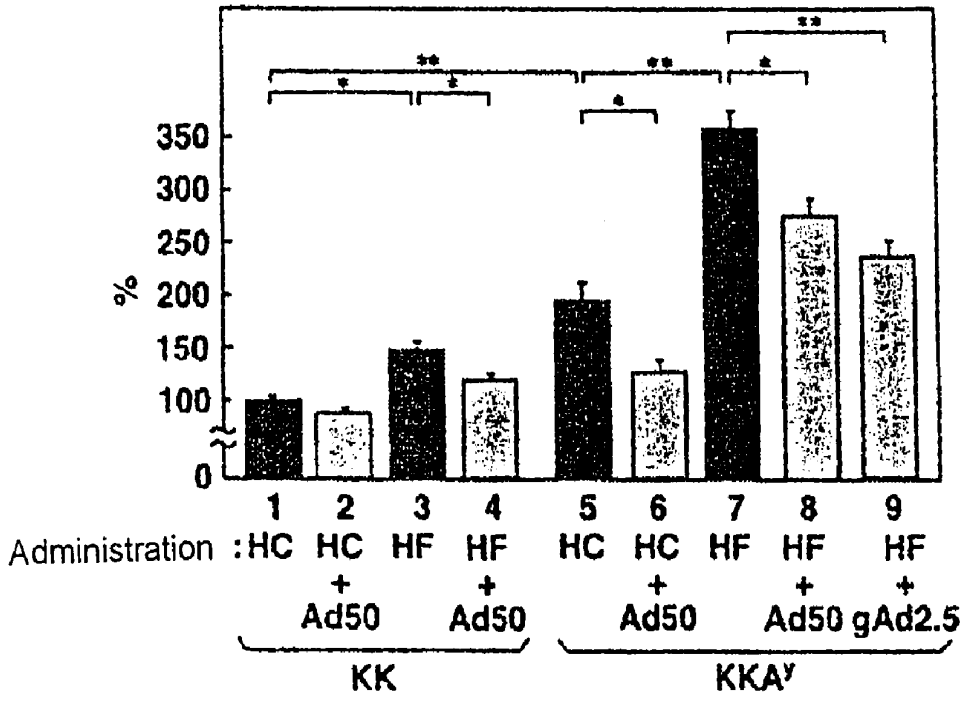

180
140
100
0
%
1 2 3 4 5 6 7 8 9
:HC HC + Ad50 HF HF + Ad50 HC HC + Ad50 HF HF + Ad50 HF + gAd2.5
Administration
KK
KKA^y

Fig. 17


%
350
300
250
200
150
100
0
1 2 3 4 5 6 7 8 9
Administration : HC HC + Ad50 HF HF + Ad50 HC HC + Ad50 HF HF + Ad50 HF + gAd2.5
KK
KKA^y

INSULIN RESISTANCE IMPROVING AGENTS

TECHNICAL FIELD

[0001] The present invention relates to an insulin resistance improving agent useful for the prevention and treatment of obesity, diabetes, and cardiovascular diseases, as well as to a drug for treating type 2 diabetes.

BACKGROUND ART

[0002] Hitherto, adipose tissue has been considered a mere storage medium for excess energy. However, recent research has elucidated that adipose tissue produces and secretes a variety of physiologically active substances. The physiologically active substances are collectively called adipocytokines, and specific members which have been known to date include leptin, tumor necrosis factor-α (TNF-α), plasminogen-activator inhibitor type 1 (PAI-1), adipsin, and resistin. Some of these adipocytokines, such as leptin, TNF-α, and resistin, have been suggested to be secreted from adipocytes to thereby control sensitivity to insulin.

[0003] Adiponectin has recently been identified as an adipocytokine. Adiponectin was originally identified independently by four research groups that used different approaches. Adiponectin cDNA was isolated by large-scale random sequencing of a 3'-directed, human-adipose-tissue cDNA library. Mouse cDNAs for adiponectin termed AcrpSO and AdipoQ were cloned through differential display before and after differentiation of mouse 3T3-L1 and 3T3-F442A cells, respectively. Human adiponectin was also purified from plasma as gelatin-binding protein 28. Obese/diabetic mice and humans exhibit significantly reduced levels of mRNA expression of adiponectin and plasma adiponectin. Lodish et al. have recently reported that a proteolytic cleavage product of Acrp30 increases fatty-acid oxidation in muscle and causes weight loss in mice.

[0004] However, whether or not adiponectin is effective in the actual treatment of diabetes remains unknown.

[0005] Insulin resistance induced by high-fat diet and accompanied with obesity is a major risk factor for diabetes and cardiovascular diseases, and therefore, capacity to improve insulin resistance is a key factor for determining that a certain drug is effective for the treatment of diabetes.

[0006] Accordingly, an object of the present invention is to provide a novel drug which improves insulin resistance and thus is useful in the treatment of diabetes.

DISCLOSURE OF THE INVENTION

[0007] The present inventors have investigated effects of adiponectin through use of model mice of different types; i.e., mice in which insulin sensitivity had been modified, obese mice, and type 2 diabetes mice, and have found that decrease in expression or loss of expression of adiponectin is a cause for development of insulin resistance, and that administration of adiponectin or a fragment of adiponectin, or introduction of any of their genes, is effective for the treatment of insulin-resistant diabetes and type 2 diabetes, thereby leading to completion of the invention.

[0008] Accordingly, the present invention provides an insulin resistance improving agent containing, as an active component, a C-terminal globular region, adiponectin, or a gene for the globular region or adiponectin.

[0009] The present invention also provides a therapeutic drug for type 2 diabetes, containing, as an active component, a C-terminal globular region, adiponectin, or a gene for the globular region or adiponectin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. **1** shows the amounts of adiponectin mRNA in WAT (a) and serum adiponectin levels (b) of db/db mice.

[0011] FIG. **2** shows the calculated areas under (a) the glucose curves and (b) the insulin curves obtained through a glucose tolerance test of db/db mice.

[0012] FIG. **3** shows the amounts of adiponectin mRNA in 3T3L1 adipocytes.

[0013] FIG. **4** shows the amounts of LPL mRNA in WAT.

[0014] FIG. **5** is a graph showing the epididymal WAT weight.

[0015] FIG. **6** shows images of the abdominal cavities of mice, which show loss of WAT.

[0016] FIG. **7** shows the results of immunoblotting through use of anti-adiponectin antibody.

[0017] FIG. **8** shows insulin resistance indices.

[0018] FIG. **9** shows expression of mRNAs of CD36, ACO, UCP2, and PPAR-α in the mouse skeletal muscle.

[0019] FIG. **10** shows expression of mRNAs of CD36, ACO, UCP2, and PPAR-α in the mouse liver.

[0020] FIG. **11** shows insulin-induced tyrosine phosphorylation and insulin(Ins)-stimulated phosphorylation of Akt of insulin receptor (IR) and insulin receptor substrate (IRS)-1 in the mouse skeletal muscle.

[0021] FIG. **12** shows serum adiponectin levels obtained through a glucose tolerance test of C57 and db/db mice.

[0022] FIG. **13** shows the calculated areas under the glucose curves obtained through glucose tolerance test of C57 and db/db mice.

[0023] FIG. **14** shows calculated areas under the insulin curves obtained through glucose tolerance test of C57 and db/db mice.

[0024] FIG. **15** shows serum adiponectin levels obtained through a glucose tolerance test of KK and KKA^y mice.

[0025] FIG. **16** shows calculated areas under the glucose curves obtained through a glucose tolerance test of KK and KKA^y mice.

[0026] FIG. **17** shows calculated areas under the insulin curves obtained through a glucose tolerance test of KK and KKA^y mice.

BEST MODE FOR CARRYING OUT THE INVENTION

[0027] Adiponectin to be used in the present invention has already been cloned [Maeda, K. et al., Biochem. Biophys. Res. Commun. 221, 286-296 (1996), Nakano, Y. et al., J. Biochem. (Tokyo) 120, 802-812 (1996)], and therefore, is available through known means. SEQ ID NOs: 1 and 2 show the amino acid sequence and the nucleotide sequence of human adiponectin, respectively. Adiponectin consists of an N-terminal collagen-like domain (cAd) and a C-terminal globular domain (gAd; in SEQ ID NO: 1, amino acid Nos. 114 to 239 or 111 to 242). The C-terminal globular domain (gAd) is highly preferred, as it provides stronger effect in alleviating high blood sugar and hyperinsulinemia. SEQ ID NOs: 3 and 4 show the amino acid sequence and nucleotide sequence of mouse adiponectin, respectively. The cAd domain of mouse adiponectin extends from the 45th to 109th amino acid residues, and the gAd domain of the same extends from the 110th to 247th amino acid residues. Also, proteins

that can be employed in the present invention are not limited to a protein having any of amino acid sequences of SEQ ID NOs: 1 to 4 or a protein having an amino acid sequence exhibiting the gAd domain; any other protein may be employed, even though it is a protein derived therefrom through substitution, deletion, or addition of one or more amino acid residues, so long as it exhibits effects equivalent to those of adiponectin. Examples of the amino acid sequence derived through substitution, deletion, or addition of one or more amino acid residues in the amino acid sequence include those sequences having 80% or more homology, more preferably 90% or more homology, to the sequence of SEQ ID NO: 1.

[0028] Examples of genes which may be used in the present invention include a gene encoding adiponectin of SEQ ID NO: 1, and a gene encoding gAd. Moreover, genes having a nucleotide sequence capable of being hybridized with any of these genes under stringent conditions may also be used.

[0029] A polypeptide of adiponectin or a portion thereof may be separated from the cells containing it. However, since a cloned gene capable of encoding adiponectin has already become available, the polypeptide may be prepared by means of the DNA recombinant technique. Specifically, an expression vector is prepared by use of the gene, and the vector is used to create transformant cells.

[0030] As shown in the Examples provided hereinbelow, model mice in which insulin sensitivity had been modified were found to exhibit a reduction in expression of adiponectin and development of insulin resistance simultaneously. Adiponectin reduces insulin resistance by lowering the triglyceride content of the muscles and the liver of an obese mouse. This mechanism is based on an elevated expression of a molecule which participates in both burning of fatty acids and energy consumption in the muscles. Also, the insulin resistance in lipoatrophic mice was alleviated by single use of either adiponectin or leptin. However, when adiponectin and leptin were used in combination, full alleviation was attained. In any of obese model mice and lipoatrophic model mice, reduced adiponectin participates in the manifestation of insulin resistance. Therefore, adiponectin has thus been proven to serve as a new type of remedy for alleviation of insulin resistance and treatment of type 2 diabetes.

[0031] When the drug of the present invention is administered to mammals including humans, a pharmacologically acceptable carrier may be added to the aforementioned active component, thereby forming pharmaceutical compositions suitable for different manners of administration. A preferred manner of administration is injection. Examples of the pharmacologically acceptable carrier include distilled water, a solubilizer, a stabilizer, an emulsifier, and a buffer. The dose of any of the drugs differs depending on the pathological condition, sex, body weight, etc. of the patient, and may be approximately 0.1 μg to 10 mg/day as reduced to the amount of adiponectin.

EXAMPLES

[0032] The present invention will next be described in more detail by way of Examples, which should not be construed as limiting the invention thereto.

A. Methods

(1) Chemicals

[0033] Rosiglitazone (PPAR-γ agonist) and HX531 (PPAR-γ/RXR inhibitor) were synthesized as described in the literature (Chem. Pharm. Bull. (Tokyo) 47, 1778-1786 (1999), Diabetes 47, 1841-1847 (1998)).

(2) Animals

[0034] PPAR-$\gamma^{+/-}$ mice were prepared in a manner which had already been reported (Mol. Cell. 4, 597-609 (1999)). All other animals were purchased from Nippon CREA. Six-week-old mice were fed powdered chow, and drugs were given as feed admixtures as described (Mol. Cell. 4, 597-609 (1999)).

(3) RNA Preparation, Northern-Blot Analysis, and Immunoblotting

[0035] Total RNA was prepared from tissues through use of TRIzol (GIBCO-BRL) according to the manufacturer's instructions. RNA obtained from 5 to 10 mice in each group was pooled, and aliquots were subjected to northern blot analysis with the probes for rat ACO (from T. Hashimoto), mouse CD36, UCP2, PPAR-α (from K. Motojima), or mouse adiponectin cDNA (J. Biol. Chem. 273, 16710-16714 (1998), Diabetes 47, 1841-1847 (1998)). The radioactivity in each band was quantified, and fold change in each mRNA was calculated after correction for loading differences by measuring the amount of 28S rRNA. Serum adiponectin levels were determined by immunoblotting with the polyclonal antibody against gelatin-binding protein 28 (raised against the peptide of CYADNDNDSTFTGFLLYHDTN, which corresponds to the C-terminal 20 amino acid residues of human adiponectin with an extra cysteine at its N terminus) through use of a recombinant adiponectin as standards (J. Biochem. (Tokyo) 120, 802-812 (1996)). The procedures used for immunoprecipitation and immunoblotting have been described (Mol. Cell. Biol. 16, 3074-3084 (1996)). The data from one of three independent experiments are shown as representative data.

(4) Blood Sample Assays

[0036] Plasma glucose, serum FFA, and triglyceride levels were determined through a glucose B-test, nonesterified fatty acid (NEFA) C-test, and triglyceride L-type (Wako Pure Chemicals), respectively. Plasma insulin was measured by insulin immunoassay (Morinaga Institute of Biological Science) (Diabetes 47, 1841-1847 (1998)). Leptin was assayed with an ELISA-based Quantikine M mouse leptin immunoassay kit (R&D Systems) according to the manufacturer's instructions.

(5) Generation of 6×His-Adiponectin Fusion Proteins.

[0037] Each of full-length mouse adiponectins, cAd and gAd, was introduced into the pQE-30 bacterial expression vector (Qiagen). The expression of histidine-tagged adiponectins, cAd and gAd, in JM-109 was induced by the addition of isopropyl β-thiogalactopyranoside to growth medium. Bacterial extracts were prepared using standard methods, and the fusion proteins were purified by elution by use of a nickel-ion agarose column (Diabetes 47, 1841-1847

(1998)). ActiClean Etox affinity columns (Sterogene Bioseparations) were used to remove potential endotoxin contaminations.

(6) Administration of Adiponectin or Leptin

[0038] Adiponectin or leptin was administered to mice through intraperitoneal injection or continuous systemic infusion as described (Nature 401, 73-76 (1999)). An Alzet micro-osmotic pump (model 1002, Alza) was inserted subcutaneously in the back of each mouse. A daily dose (shown in Figures) of recombinant leptin (Sigma) or adiponectin was dissolved in a total volume of 0.1 mL of PBS, and the solution was delivered to mice through the pump for twelve days.

(7) Insulin-Resistance Index

[0039] The areas of glucose and insulin curves were calculated by multiplying the cumulative mean height of the glucose values (1 mg/ml=1 cm) and insulin values (1 ng/ml=1 cm), respectively, by time (60 min=1 cm) (Am. J. Physiol. 240, E482-488 (1981)). The insulin resistance index was calculated from the product of the areas of glucose and insulin$\times 10^{-2}$ in glucose tolerance test (Mol. Cell. 4, 597-609 (1999)). The results are expressed as the percentage of the value of each control.

(8) Lipid Metabolism and Enzymatic Activity of ACO

[0040] The measurements of [^{14}C] CO_2 production from [1-^{14}C] palmitic acid were performed using liver and muscle slices as described (Diabetes 47, 1841-1847 (1998)). Liver and muscle homogenates were prepared, and tissue triglyceride content was determined with an extract solution ($CHCl_3$:CH_3OH=2:1). The remainder of the liver and muscle was immediately frozen in liquid nitrogen and stored at −80° C. until measurement of the enzymatic activity of ACO. ACO activity in the light mitochondrial fraction of liver and muscle was measured by assay that was based on the H_2O_2 dependent oxidation of leuco-dichlorofluorescein (Diabetes 47, 1841-1847 (1998)).

B. Results

(1) Relationship Between Adiponectin Expression and Insulin Sensitivity

[0041] Because adiponectin is reported to be decreased in obesity, we investigated the role of altered adiponectin expression in obesity and insulin resistance. To this end, we used the PPAR-γ agonist, rosiglitazone, which promotes adipogenesis and reduces insulin resistance.

[0042] The results are shown in FIGS. **1**, **2**, and **3**. FIG. **1** shows amounts of the adiponectin mRNA in WAT (FIG. **1***a*) or serum levels of adiponectin (FIG. **1***b*) of db/db mice on the high-carbohydrate diet (HC), on the high-fat diet (HF), or on the high-fat diet and treated with rosiglitazone (HF+Rosi).

[0043] FIG. **2** shows values of area under the glucose curve (FIG. **2***a*) and area under the insulin curve (FIG. **2***b*) obtained through a glucose tolerance test of db/db mice which had been subjected to the high-carbohydrate diet (HC), to the high-fat diet (HF), or to the high-fat diet and treated with rosiglitazone (HF+Rosi). Results are expressed as the percentage of the value based on that of untreated mice on the HC diet. The basal glucose levels (time=0 of the glucose tolerance test) of untreated db/db mice on the HC diet were 244.8±23.3 mg/dl (FIG. **2***a*). Each bar represents the mean±s.e. (n=5-10) (*, P<0.05; **, P<0.01; compared with untreated mice on the HC diet).

[0044] FIG. **3** shows amounts of adiponectin mRNA in 3T3L1 adipocytes which were untreated (−) or treated with 1 μM rosiglitazone (Rosi) for 24 hours.

[0045] A high-fat diet reduced the mRNA levels in white adipose tissue (WAT) (FIG. **1***a*) and serum levels of adiponectin (FIG. **1***b*) in mice with hyperglycemia (FIG. **2***a*) and hyperinsulinemia (FIG. **2***b*). Rosiglitazone significantly increased the mRNA levels in WAT (FIG. **1***a*) and serum levels of adiponectin (FIG. **1***b*) in mice on high-fat diet, and, at the same time, ameliorated hyperglycemia (FIG. **2***a*) and hyperinsulinemia (FIG. **2***b*). There was, however, a slight increase in adipose tissue mass (vehicle: 2.72±0.11 g; Rosi: 2.84±0.16 g) and body weight (vehicle: 46.5±0.70 g; Rosi: 47.9±1.0 g) in db/db mice. In the case of wild-type controls (C57) also, similar results were obtained. In differentiated 3T3L1 adipocytes in vitro, rosiglitazone also increased adiponectin expression (FIG. **3***c*). These data raise the possibility that the expression of adiponectin mRNA might be partially regulated by a PPAR-γ-dependent pathway, and more closely related to regulation of insulin sensitivity than obesity.

(2) Relationship Between Depletion of Adipose Tissue and Adiponectin

[0046] To clarify the causal relationship between adiponectin expression and insulin sensitivity, we attempted to deplete adipose tissue and hence adiponectin. We developed a mouse model without adipose tissue by severely reducing PPAR-γ/RXR activity (FIGS. **4** to **8**).

[0047] While PPAR-$\gamma^{+/-}$ mice were treated with HX531 for six weeks (+) or untreated (−), recombinant full-length adiponectin (Ad), gAd, or leptin (Lep) was administered to each mouse at a predetermined dose (μg/day). Unless otherwise described herein, administration was performed through continuous systemic infusion (pump) in combination with a high-fat (HF) diet for the final twelve days of the six-week HX531 treatment.

[0048] FIG. **4** shows amounts of LPL mRNA in WAT. FIG. **5** shows epididymal WAT weight. FIG. **6** presents images of the abdominal cavities of the mice illustrating loss of WAT. FIG. **7** shows serum adiponectin levels determined by immunoblotting with anti-adiponectin antibody through use of recombinant adiponectin as standards. In FIG. **7**, lane 9 shows the serum adiponectin level when 50 μg of Ad was administered to mice through intraperitoneal (ip) injection. FIG. **8** shows insulin resistance indices. The results are expressed as the percentage of the value based on that of untreated PPAR-$\gamma^{+/-}$ mice on the high-fat diet. The basal glucose level (time=0 of the glucose tolerance test) of untreated PPAR-$\gamma^{+/-}$ mice on the high-fat diet was 110.7±12.8 mg/dl. Each bar represents the mean±s.e. (n=5-10) (*, P<0.05; **, P<0.01; compared with PPAR-$\gamma^{+/-}$ mice untreated or treated with HX531 alone for six weeks or with PPAR-$\gamma^{+/-}$ mice treated with both leptin and adiponectin).

[0049] As a result, administration of a PPAR-γ/RXR inhibitor such as an RXR antagonist HX531 to PPAR-$\gamma^{+/-}$ mice for three weeks markedly lowered expression of genes responsive to PPAR-γ/RXR, such as lipoprotein lipase (LPL) in WAT (about 90% or further; FIG. **4**), indicating that PPAR-γ/RXR activity was likely to be significantly decreased. Four weeks of this treatment resulted in disappearance of visible WAT (FIGS. **5** and **6**). This loss of fat tissue presumably

results from lowered expression of molecules involved in triglyceride accumulation in WAT, the expression of which is dependent on PPAR-γ/RXR activity.

[0050] Adiponectin was completely absent in sera from the lipoatrophic mice, whereas adiponectin was detected as a 35-kD protein with an antibody against adiponectin in sera from control mice (FIG. **7**, lanes 6 and 7).

[0051] Tissue triglyceride content and free fatty acid in serum in the lipoatrophic mice were also determined.

[0052] While PPAR-$\gamma^{+/-}$ mice were treated with HX531 for six weeks (+) or untreated (−), recombinant full-length adiponectin (Ad), gAd, or leptin (Lep) was administered to each PPAR-$\gamma^{+/-}$ mouse at a predetermined dose (μg/day). Administration was performed through continuous systemic infusion in combination with the high-fat (HF) diet for the final twelve days of the six-week HX531 treatment (six weeks).

[0053] The lipoatrophic mice showed increased serum free fatty acid (FFA) levels, increased triglyceride levels, increased tissue triglyceride content in skeletal muscle and liver (Table 1) as well as hyperinsulinemia and hyperglycemia (FIG. **8**).

TABLE 1

Tissue triglyceride content and serum free fatty acid level in lipoatrophic mice

	—		HX531		
	—	Ad50	—	Ad50	gAd2.5
Skeletal muscle TG content (mg/g tissue)	6.24 ± 0.43	5.47 ± 0.32*	15.96 ± 1.47	7.74 ± 0.65**	3.88 ± 1.74**
Liver TG content (mg/g tissue)	8.02 ± 1.18	6.45 ± 0.26*	19.36 ± 1.23	16.19 ± 0.72**	13.81 ± 0.91**
Serum FFA (mEq/L)	0.42 ± 0.03	0.35 ± 0.02*	1.48 ± 0.25	0.61 ± 0.13**	0.43 ± 0.14**
Serum TG (mg/dl)	82.6 ± 8.9	60.5 ± 6.0*	201.4 ± 25.3	106.6 ± 18.1**	100.3 ± 17.5**

Mean ± s.e. (n = 5-10),
*P < 0.05,
**P < 0.01 (compared with untreated mice or mice treated with HX531 alone for 6 weeks)

(3) Reversal of Insulin Resistance of Lipoatrophic Mice by Use of Adiponectin

[0054] To determine the role of adiponectin deficiency in the development of insulin resistance in lipoatrophic mice, adiponectin was administered to the mice. Continuous systemic infusion of recombinant adiponectin at a physiological concentration (FIG. **7**, lanes 6 to 8) significantly ameliorated hyperglycemia and hyperinsulinemia (FIG. **8**).

(4) Effect of Globular Domain of Adiponectin

[0055] Adiponectin is composed of an N-terminal collagen-like sequence (cAd) and a C-terminal globular domain (gAd) (see SEQ ID NO: 1). An analysis was performed to determine which domain exerts these physiological effects. As a result, gAd ameliorated hyperglycemia and hyperinsulinemia much more potently than full-length adiponectin (FIG. **8**). A 25-kD protein recognized by an antibody against C-terminal portion of adiponectin was present in the serum, albeit in a very small amount, suggesting that full-length adiponectin might undergo proteolytic processing.

(5) Improvement of Insulin Resistance Through Adiponectin/Leptin Deficiency

[0056] Insulin resistance in lipoatrophic diabetes might be due to deficiency of adipocytokines which sensitize tissues to insulin. The above results indicate that adiponectin is one of such adipocytokines. Administration of adiponectin at a physiological concentration was not sufficient to completely ameliorate insulin resistance in mice without adipose tissue. Leptin has also been known to be such an adipocytokine. Serum leptin levels were undetectable in these mice (upper limit: 0.2 ng/ml). Administration of leptin to these mice at a physiological concentration did indeed alleviate their insulin resistance, albeit partially (FIG. **8**). Administration of adiponectin and leptin in combination at a physiological concentration almost completely removed insulin resistance synergistically (FIG. **8**).

(6) Decrease in Tissue Triglyceride Content Caused by Adiponectin

[0057] To determine the mechanism by which adiponectin exerts effects of treating diabetes, effects of adiponectin in individual organs were investigated.

[0058] FIGS. **9** and **10** show mRNAs of fatty-acid translocase (FAT)/CD36, ACO, UCP2, and PPAR-α in mouse skeletal muscle and in liver, respectively. FIG. **11** shows insulin-induced tyrosine phosphorylation of insulin receptor (IR) and insulin receptor substrate (IRS)-1 in skeletal muscle, and insulin-stimulated phosphorylation of Akt in skeletal muscle. HX531 was administered as a 0.1% food admixture. Mice were stimulated with or without 1 U/g body weight of insulin for two minutes. Lysates were subjected to immunoprecipitation (IP) and immunoblotting through use of the antibodies described in Mol. Cell. Biol. 16, 1074-3084 (1996).

[0059] As a result, in skeletal muscle, administration of gAd at a low concentration increased expression of molecules involved in fatty-acid transport, combustion and energy dissipation such as CD36, acyl-CoA oxidase (ACO), and uncoupling protein $(UCP)_2$, respectively (FIG. **9**). In turn, these processes lead to decreased tissue triglyceride content in skeletal muscle (Table 1). Expression of these genes has been known to be positively controlled by PPAR. Therefore, possible mechanism(s) underlying these alterations in gene expression might include increased expression of PPAR-α/γ and/or increased endogenous ligands. Indeed, expression of PPAR-α was increased (FIG. **9**). In contrast to skeletal muscle, in the liver, administration of gAd at a low concentration decreased the expression of molecules involved in

fatty-acid transport into tissues such as CD36 (FIG. **10**), thereby reducing fatty-acid influx into the liver, which might lead to decrease in hepatic triglyceride content in the liver (Table 1). Thus, even though triglyceride content was decreased in both muscle and liver, the mechanisms involved in the decrease in triglyceride content are apparently quite different in the two tissues. These data indicate that adiponectin acts primarily on skeletal muscle to increase influx and combustion of FFA, thereby reducing muscle triglyceride content. As a consequence of decreased serum FFA and triglyceride levels (Table 1), hepatic triglyceride content is decreased.

(7) Improvement of Insulin Signaling Caused by Decrease of Triglyceride Content

[0060] Increased tissue triglyceride content has been reported to interfere with insulin-stimulated activation of phosphatidylinositol-3-kinase and subsequent translocation of glucose-transporter protein 4 to surfaces of cell membrane and uptake of glucose, which leads to development of insulin resistance. Thus, decreased triglyceride content in muscle might contribute to the improved insulin signal transduction, as demonstrated by increase in insulin-induced tyrosine phosphorylation of insulin receptor and insulin-receptor substrate 1, as well as increases in insulin-stimulated phosphorylation of Akt kinase in skeletal muscle of adiponectin-administered lipoatrophic mice (FIG. **11**).

(8) Effect of Adiponectin on Improvement of Insulin resistance in obese mice

[0061] Next, studies were performed to investigate whether adiponectin can improve insulin resistance and diabetes in db/db and KKA^y mice (KK mice overexpressing agouti), two different mouse models of type 2 diabetes characterized by obesity, hyperlipidemia, insulin resistance, and hyperglycemia.

[0062] The results are shown in FIGS. **12** to **17**: serum levels of adiponectin (FIGS. **12** and **15**), areas under the glucose curve (FIGS. **13** and **16**), and areas under the insulin curve (FIGS. **14** and **17**), obtained through glucose tolerance test (GTT) of C57 or db/db mice (FIGS. **12** to **14**) or of KK or KKA^y mice (FIGS. **15** to **17**). The mice were fed an HC or HF diet. In addition, Ad or gAd was administered, or none of these was administered, to the mice at a predetermined dose (μg/day). Serum adiponectin levels were determined by immunoblotting with anti-adiponectin antibody through use of a recombinant adiponectin as standards (FIGS. **12** and **15**). The results are expressed as the percentage of the value based on untreated wild-type mice on the HC diet (FIGS. **13**, **14**, **16**, and **17**). The basal glucose level (time=0 of the GTT) of untreated C57 mice on the HC diet was found to be 62.3±3.1 mg/dl (FIG. **13**), and that of KK mice was found to be 93.0±6.1 mg/dl (FIG. **16**). Each bar represents the mean±s.e. (n=5-10) (*, P<0.05; **, P<0.01; C57 versus db/db, KK versus KKA^y, or HC versus HF, or compared with untreated mice).

[0063] As a result, serum adiponectin levels were decreased in wild-type mice on a high-fat diet (FIG. **12**, lane 3) as compared with those in mice on a high-carbohydrate diet (FIG. **12**, lane 1). Serum adiponectin levels in db/db mice (FIG. **12**, lanes 5 and 7) were also decreased as compared with wild-type controls on either high-carbohydrate or high-fat diet (FIG. **12**, lanes 1 and 3). Lower serum adiponectin levels in wild-type mice on the high-fat diet were partially restored to those of wild-type controls on the high-carbohydrate diet by continuous systemic infusion of low doses of recombinant adiponectin (FIG. **12**, lanes 1, 3, and 4). The infusion also significantly ameliorated hyperglycemia and hyperinsulinemia (FIG. **14**, lanes 1, 3, and 4) induced by high-fat diet (FIG. **13**, lanes 1, 3, and 4). Lower serum adiponectin levels in db/db mice on either high-carbohydrate or high-fat diet were also partially restored to those in respective wild-type controls by the adiponectin replenishment (FIG. **12**), which also significantly ameliorated leptin receptor deficiency-induced hyperglycemia (FIG. **13**) and hyperinsulinemia (FIG. **14**). We obtained similar results when using KKA^y mice and their wild-type controls (FIGS. **15** to **17**). These data indicate that high-fat feeding, leptin-receptor deficiency, or agouti overexpression causes insulin resistance, partially through decreases in adiponectin, and that adiponectin is useful as an anti-diabetic drug.

[0064] In addition, effect of administration of adiponectin on fatty acid oxidation in the skeletal muscle of KKA^y mice was studied.

[0065] The following values were determined through use of KKA^y mice: acyl-CoA oxidase (ACO) activity and fatty acid oxidation in the skeletal muscle and in the liver, tissue triglyceride content in the skeletal muscle and in the liver, and serum free fatty acid level and serum triglyceride level. Mice were fed with high fat diet, and full-length adiponectin (Ad) or adiponectin globular domain (gAd) was administered to each mice at a dose shown in Table 2 for two weeks.

[0066] The results are shown in Table 2.

TABLE 2

		—	Ad50	gAd2.5
ACO activity	skeletal muscle	0.24 ± 0.02	0.37 ± 0.04*	0.42 ± 0.04**
(nmol/mg/min)	liver	3.21 ± 0.33	3.04 ± 0.85	2.89 ± 0.35
Fatty acid oxidation	skeletal muscle	2.52 ± 0.23	3.95 ± 0.58*	4.06 ± 0.44*
[^{14}C] palmitate→CO_2	liver	3.31 ± 0.38	2.92 ± 0.29	2.89 ± 0.21
(nmol/g/h)				
TG content	skeletal muscle	10.94 ± 1.03	8.75 ± 0.58*	8.06 ± 0.61*
(mg/g tissue)	liver	19.07 ± 1.78	16.15 ± 0.83*	16.04 ± 0.91*
Serum FFA (mEq/L)		1.29 ± 0.12	0.67 ± 0.09**	0.39 ± 0.04**
Serum TG (mg/dl)		200.2 ± 20.8	101.3 ± 19.7**	96.4 ± 18.3**
Rectal temperature (° C.)		36.7 ± 0.3	37.3 ± 0.2*	37.7 ± 0.1**

Mean ± s.e. (n = 5-10),
*P < 0.05,
**P < 0.01 (compared with untreated mice)

(9) Facilitation of β-Oxidation by Adiponectin

[0067] In skeletal muscle, adiponectin-administered KKA^{y} mice showed increased expression of enzymes involved in β-oxidation and UCP2. In mice to which adiponectin had been administered, ACO activities and fatty-acid combustion were increased in skeletal muscle but not liver (Table 2). These alterations decreased triglyceride content in skeletal muscle, and also decreased serum FFA and triglyceride levels (Table 2). These reductions in serum FFA and triglyceride levels seem to cause subsequent decreased expression of molecules involved in fatty-acid transport into hepatic tissues, thereby also reducing tissue triglyceride content in liver (Table 2).

[0068] In contrast, administration of adiponectin to normal C57 mice for two weeks slightly, but not significantly, reduced the increases in WAT weight (untreated mice: 0.53±0.03 g; gAd-treated: 0.48±0.04 g) and body weight associated with the high-fat diet as compared with vehicle (vehicle-treated: 22.8±2.0 g; gAd-treated: 20.6±2.1 g). Food intake tended to be higher in adiponectin-treated mice as compared with the control on the high-fat diet (vehicle-treated: 5.71±0.56 g/day; gAd-treated: 6.28±0.51 g/day), and the rectal temperature was significantly higher in mice to which adiponectin had been administered (Table 2), consistent with increased expression of molecules involved in fatty-acid combustion and energy dissipation in muscle and brown adipose tissue. However, the anti-diabetic effects of adiponectin were not attenuated in db/db mice, which lack leptin receptor (FIGS. **12** to **14**). Moreover, administration of adiponectin to wild-type mice did not alter the expression of leptin in WAT and serum leptin levels (vehicle: 11.1±2.1 ng/ml; gAd: 10.4±2.6 ng/ml). We obtained essentially similar results of serum leptin levels by using KK (vehicle: 15.1±2.5 ng/ml; gAd-treated: 13.4±2.7 ng/ml), KKA^{y} (vehicle: 61.5±5.4 ng/ml; gAd-treated: 57.9±5.7 ng/ml), and db/db mice (vehicle: 153.9±20.4 ng/ml; gAd-treated: 145.2±14.7 ng/ml). These findings indicate that adiponectin exerted effects of treating diabetic through leptin-independent pathways.

INDUSTRIAL APPLICABILITY

[0069] The present invention reverses insulin resistance induced from a high fat diet and associated with obesity, and therefore, enables treatment of type 2 diabetes, which is the most common among other types of diabetes.

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Ile Gln Gly Arg Lys Gly Glu Pro Gly Glu Gly Ala Tyr Val Tyr Arg
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Ser Ala Phe Ser Val Gly Leu Glu Thr Tyr Val Thr Ile Pro Asn Met
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85                  90                  95                  100

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Gly Glu Thr Gly Asp Val Gly Met Thr Gly Ala Glu Gly Pro Arg Gly
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Phe Pro Gly Thr Pro Gly Arg Lys Gly Glu Pro Gly Glu Ala Ala Tyr
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Tyr Tyr Phe Ser Tyr His Ile Thr Val Tyr Met Lys Asp Val Lys Val
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Ser Leu Phe Lys Lys Asp Lys Ala Val Leu Phe Thr Tyr Asp Gln Tyr
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Leu Leu Tyr His Asp Thr Asn
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What we claim is:

1. A method for improving insulin resistance in a subject in need thereof comprising administering an effective amount of an agent containing, as an active component, a C-terminal globular domain of adiponectin.

2. The method of claim **1**, wherein said agent further comprises a pharmacologically acceptable carrier.

3. The method of claim **2**, wherein said pharmacologically acceptable carrier is selected from the group consisting of distilled water, a solubilizer, a stabilizer, an emulsifier, and a buffer.

4. The method of claim **1**, wherein said administering is by injection.

5. The method of claim **1**, wherein said effective amount ranges from 0.1 μg/day to 10 mg/day based on the amount of adiponectin.

6. The method of claim **1**, wherein said C-terminal globular domain of adiponectin encompasses amino acid residues 111 to 242 of SEQ ID NO: 1 or 114 to 239 of SEQ ID NO: 1.

7. The method of claim **1**, wherein said C-terminal globular domain of adiponectin encompasses amino acid residues 114 to 239 of SEQ ID NO: 1.

8. A method for treating type 2 diabetes in a subject in need thereof comprising administering an effective amount of a therapeutic agent for type 2 diabetes containing, as an active component, a C-terminal globular domain of adiponectin.

9. The method of claim **8**, wherein said agent further comprises a pharmacologically acceptable carrier.

10. The method of claim **9**, wherein said pharmacologically acceptable carrier is selected from the group consisting of distilled water, a solubilizer, a stabilizer, an emulsifier, and a buffer.

11. The method of claim **8**, wherein said administering is by injection.

12. The method of claim **8**, wherein said effective amount ranges from 0.1 μg/day to 10 mg/day based on the amount of adiponectin.

13. The method of claim **8**, wherein said C-terminal globular domain of adiponectin encompasses amino acid residues 111 to 242 of SEQ ID NO: 1 or 114 to 239 of SEQ ID NO: 1.

14. The method of claim **8**, wherein said C-terminal globular domain of adiponectin encompasses amino acid residues 114 to 239 of SEQ ID NO: 1.

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