

The hormone resistin links obesity to diabetes

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Diabetes mellitus is a chronic disease that leads to complications including heart disease, stroke, kidney failure, blindness and nerve damage. Type 2 diabetes, characterized by target-tissue resistance to insulin, is epidemic in industrialized societies and is strongly associated with obesity; however, the mechanism by which increased adiposity causes insulin resistance is unclear. Here we show that adipocytes secrete a unique signalling molecule, which we have named resistin (for resistance to insulin). Circulating resistin levels are decreased by the anti-diabetic drug rosiglitazone, and increased in diet-induced and genetic forms of obesity. Administration of anti-resistin antibody improves blood sugar and insulin action in mice with diet-induced obesity. Moreover, treatment of normal mice with recombinant resistin impairs glucose tolerance and insulin action. Insulin-stimulated glucose uptake by adipocytes is enhanced by neutralization of resistin and is reduced by resistin treatment. Resistin is thus a hormone that potentially links obesity to diabetes.

Diabetes mellitus is a principal cause of morbidity and mortality in human populations. A minority of patients suffer from type 1 diabetes mellitus, which is caused by pancreatic β -cell failure and leads to an absolute loss of insulin¹. Type 2 diabetes mellitus is far more common, and is swiftly increasing in prevalence in industrialized societies. Type 2 diabetes mellitus is characterized by target-tissue resistance to insulin that cannot be overcome by β -cell hypersecretion². Although type 2 diabetes mellitus is strongly associated with insulin resistance in both human and rodent models of type 2 diabetes mellitus³, the connection between increased adiposity and insulin resistance remains unknown⁴. Fat-cell secretion of free fatty acids may contribute to insulin resistance in peripheral tissues⁵. Adipocytes also secrete a variety of polypeptides, such as leptin, tumour necrosis factor- α (TNF- α), adipon and Acrp30/adipoQ, that may affect insulin action in other tissues^{6–11}.

A new class of anti-diabetic drugs called thiazolidinediones (TZDs) enhance target-tissue sensitivity to insulin *in vivo*¹² where they function as high-affinity ligands for a nuclear receptor, particularly abundant in fat cells, called peroxisome proliferator activated receptor- γ (PPAR γ)^{13–15}. The best understood function of PPAR γ is as an adipogenic determination factor^{16–18}. However, it is probable that PPAR γ is the biological target of the anti-diabetic actions of TZDs: there is a strong correlation between PPAR γ binding *in vitro* and glucose lowering *in vivo*¹⁹; non-TZD PPAR γ ligands also increase insulin sensitivity¹⁹; activators of the PPAR γ heterodimer partner, retinoid X receptor, also have anti-diabetic properties²⁰; and patients heterozygous for a dominant-negative PPAR γ allele have severe insulin resistance, providing a genetic link between PPAR γ and diabetes²¹. Notably, mice heterozygous for a null PPAR γ allele develop adipocyte hypertrophy on a high-fat diet and have increased sensitivity to insulin^{22,23}. The gene targets of TZD-bound PPAR γ that regulate insulin sensitivity are unknown.

We thought that insulin resistance might be mediated by a TZD-regulated adipocyte-derived factor. A screen for genes that are induced during adipocyte differentiation but downregulated in mature adipocytes exposed to TZDs led to the discovery of a protein that we have called resistin (Fig. 1a). Resistin gene expression is induced during adipocyte differentiation, and the resistin polypeptide is specifically expressed and secreted by adipocytes. Resistin circulates in mouse serum, and its level is increased markedly in both genetic and diet-induced obesity. Immunoneutralization

improves blood glucose and insulin action in this model of type 2 diabetes. By contrast, administration of resistin impairs glucose tolerance and insulin action in normal mice. Resistin is therefore a strong candidate to explain the anti-diabetic effects of TZDs, as well as a mechanism by which excess adiposity leads to insulin resistance.

Resistin is a TZD-regulated protein

We considered that TZD treatment downregulates an adipocyte gene that contributes to insulin resistance. Resistin messenger RNA is induced markedly during adipocyte differentiation of 3T3-L1 cells, similar to other adipocyte-specific genes, such as PPAR γ (Fig. 1b). Notably, resistin gene expression was markedly downregulated by treatment with the TZD rosiglitazone, unlike the adipocyte-specific protein aP2, a well-characterized PPAR γ target gene¹⁴ (Fig. 1c). Other TZDs, including pioglitazone and troglitazone, also downregulated resistin (data not shown).

A notable feature of the deduced amino-acid sequence of resistin is a hydrophobic amino terminus that is predicted by the Prosort³⁴ and SignalP³⁵ algorithms to be a functional export signal (Fig. 1a). Resistin was abundant in the media of transfected 293T cells, and N-terminal Edman sequencing showed that the protein begins at Ser 21 of the deduced amino-acid sequence (data not shown). This suggests that resistin is specifically processed and secreted. Of note, endogenous resistin was secreted into the media by 3T3-L1 adipocytes (Fig. 1d). Moreover, consistent with its gene expression, resistin secretion was reduced markedly after exposure of the adipocytes to rosiglitazone for 4 d (Fig. 1e).

Adipocyte-specific expression of resistin

We next evaluated resistin gene expression in the mouse. A single mRNA of roughly 750 residues was robustly expressed in white adipose tissue but not in several other mouse tissues (Fig. 2a and b). Resistin expression was greater in white adipose tissue than in brown adipose tissue, where resistin mRNA was barely detectable (Fig. 2b). A low level of resistin expression was detected in mammary tissue, which is potentially due to the presence of the mammary fat pad (Fig. 2a). Resistin mRNA levels varied as a function of white adipose depot and gender, with the highest level of expression in female gonadal fat (Fig. 2b). Immunohistochemistry of epididymal white adipose tissue showed that the resistin protein was abundant in adipocyte cytoplasm (Fig. 2c). We have

also identified a human expressed sequence tag that is highly related to resistin. The deduced amino-acid sequence includes an N-terminal signal sequence and strict conservation of cysteine residues (Fig. 2d). The unique pattern of cysteines (X₁₁-C-X₈-C-X-C-X₃-C-X₁₀-C-X-C-X-C-X₉-CC-X₃₋₆-END) is conserved in a family of resistin-like molecules including at least three distinct mouse subtypes^{24,25}. The human resistin homologue is also secreted after transfection into 293T cells (data not shown). We showed adipocyte expression of the putative human resistin by using polymerase chain reaction with reverse transcription (RT-PCR) analysis (data not shown). The coding region of the entire human resistin gene was localized to a cloned fragment of human chromosome 19 (accession number AC008763, data not shown).

Downregulation of resistin

We analysed serum resistin levels, as resistin is a secreted protein produced by adipocytes. Notably, immunoreactive resistin was readily detectable in serum from normal mice (Fig. 3a). Resistin was also detectable in normal rat serum (data not shown). Mouse serum levels decreased after a 48-h fast, and were reversed by refeeding (Fig. 3a). Resistin gene and protein expression were both downregulated in epididymal fat (Fig. 3b, c). Moreover, consistent with the *in vitro* results, rosiglitazone treatment markedly lowered serum resistin levels in mice (Fig. 3d).

Diet-induced and genetically obese mice

Resistin was identified in a search for an adipocyte signal that mediated target tissue insulin resistance. We therefore analysed serum resistin levels in mice that developed obesity and insulin resistance on a high-fat diet²⁶. Resistin serum levels were markedly elevated in mice fed on a diet containing 45% (by kJ) of fat for eight weeks (Fig. 4a). The increase in serum resistin was observed within four weeks of high-fat feeding, as mice became insulin resistant and obese (Fig. 4b). Serum resistin levels were also elevated in *ob/ob* and *db/db* mice in which obesity and diabetes are inherited traits (Fig. 4c).

Neutralization of resistin

We next considered whether the increased level of resistin in mice

with diet-induced obesity was causally related to the associated type 2 diabetes. As antibodies to other hormones and signalling molecules can neutralize their function, we tested the effects of administering anti-resistin immunoglobulin-γ (IgG) on blood glucose in mice with diet-induced obesity, insulin resistance and hyperglycaemia (mean glucose 1.63 ± 0.10 mg ml⁻¹). Treatment of mice with nonspecific IgG had no significant effect on blood glucose (Fig. 5a). By contrast, treatment of mice with an equivalent amount of IgG purified from anti-resistin antiserum caused a significant decrease in blood glucose (1.37 ± 0.98 mg ml⁻¹, $P = 0.02$). After IgG therapy, blood glucose returned to levels indistinguishable from those before the experiment (Fig. 5b). Neutralization of resistin reversibly reduced hyperglycaemia in this model of diet-induced insulin resistance. To investigate the mechanism governing this effect, insulin tolerance tests were performed after treatment with control or anti-resistin IgG. Mice treated with anti-resistin IgG showed significantly improved sensitivity to insulin compared with control IgG treatment (Fig. 5b). These data indicate that resistin may be involved in mediating insulin resistance associated with diet-induced obesity.

Resistin impairs glucose tolerance *in vivo*

To investigate resistin function *in vivo*, recombinant resistin was expressed with a carboxy-terminal Flag-tag (resistin-F) in 293T cells, and affinity purified from the medium to apparent homogeneity (Fig. 6a). This preparation, containing less than 1 endotoxin unit per ml, was used for *in vivo* studies. Serum resistin concentration increased 15 min after intraperitoneal (i.p.) administration of 16.5 μg purified, recombinant resistin-F (Fig. 6b). Resistin levels were maximally elevated at 30–60 min, and reduced but still elevated 4 h after i.p. administration (Fig. 6b).

We next measured glucose tolerance after i.p. administration of resistin-F. Purified resistin-F (16.5 μg i.p.) or vehicle was administered to C57Bl/6J mice at the beginning of an overnight fast and again 12 h later. We measured glucose tolerance 2 h after the second injection of resistin-F. Peak blood glucose increased by 28% ($P = 0.004$) in the resistin-treated mice (Fig. 6c). Insulin levels were increased concomitantly with the increased blood glucose in resistin-F-treated mice (data not shown). Although this

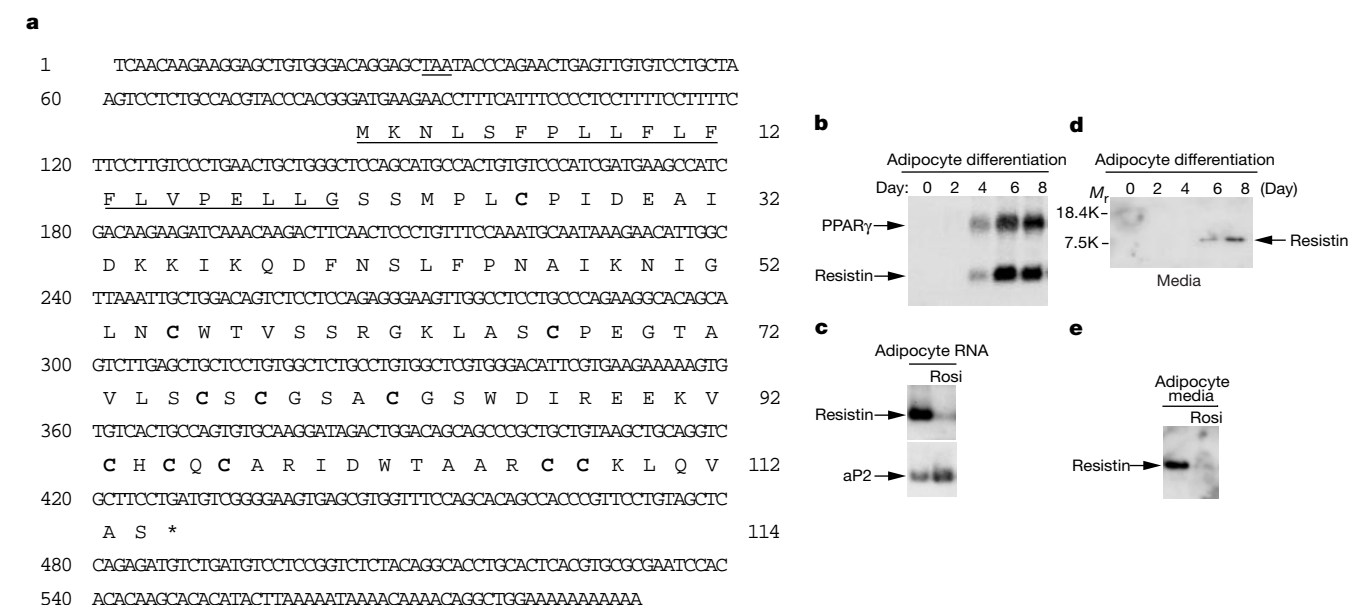


Figure 1 Cloning and identification of resistin. **a**, Resistin cDNA and amino-acid sequence. Stop codon, polyadenylation sequences and putative signal sequence are underlined. Asterisk indicates stop codon; cysteine residues are shown in bold. **b**, Northern analysis of resistin and PPARγ gene expression during adipogenic

differentiation of 3T3-L1 cells. **c**, Downregulation of resistin gene expression in 3T3-L1 adipocytes by rosiglitazone (Rosi, 1 μM). Expression of aP2 is shown for comparison. **d**, Resistin protein is induced and secreted during adipogenesis. **e**, Adipocyte resistin secretion is markedly reduced by rosiglitazone (Rosi, 1 μM).

increase was not statistically significant, the observation that insulin levels were not decreased after resistin-F administration indicates that the glucose intolerance may be due to insulin resistance rather than failure to produce or secrete insulin. The hypoglycaemic response to insulin was blunted significantly in resistin-F-treated mice (Fig. 6d). We cannot rule out the possibility that the Flag epitope altered resistin activity; however, conditioned medium from 293T cells transfected with wild-type resistin—but not conditioned medium from vector-transfected cells—also produced glucose intolerance when injected into mice (data not shown). Moreover, the ability of resistin-F to produce glucose intolerance and insulin resistance is consistent with the opposite effects of immunoneutralization of endogenous resistin.

Resistin antagonizes insulin action *in vitro*

3T3-L1 adipocytes are commonly used as a model system to study insulin-stimulated glucose uptake. Our observation that 3T3-L1 cells secrete abundant resistin into their medium suggests that this factor may modulate insulin-stimulated glucose transport in these

cultured cells through an autocrine or paracrine mechanism. We exposed 3T3-L1 adipocytes to IgG purified from resistin antiserum, and measured the uptake of [^3H]2-deoxyglucose in the basal state and after stimulation by insulin (5 nM). Anti-resistin IgG potentiated insulin-stimulated glucose uptake by 42% ($P < 0.04$), whereas control IgG had little effect (Fig. 7a). Similar effects of resistin antiserum (stimulation range 15–100%) were observed in several experiments using insulin at concentrations of 5–100 nM (data not shown). Consistent with these results, treatment of 3T3-L1 adipocytes with purified resistin-F reduced insulin-stimulated glucose uptake by 37% ($P < 0.02$) in 3T3-L1 adipocytes (Fig. 7b). Together, these data suggest that resistin functions as a signal to decrease insulin-stimulated glucose uptake in this model of insulin action.

Discussion

Resistin is a signalling molecule that is induced during adipogenesis and secreted by adipocytes. Resistin gene expression and protein secretion are markedly reduced by anti-diabetic TZDs. Resistin

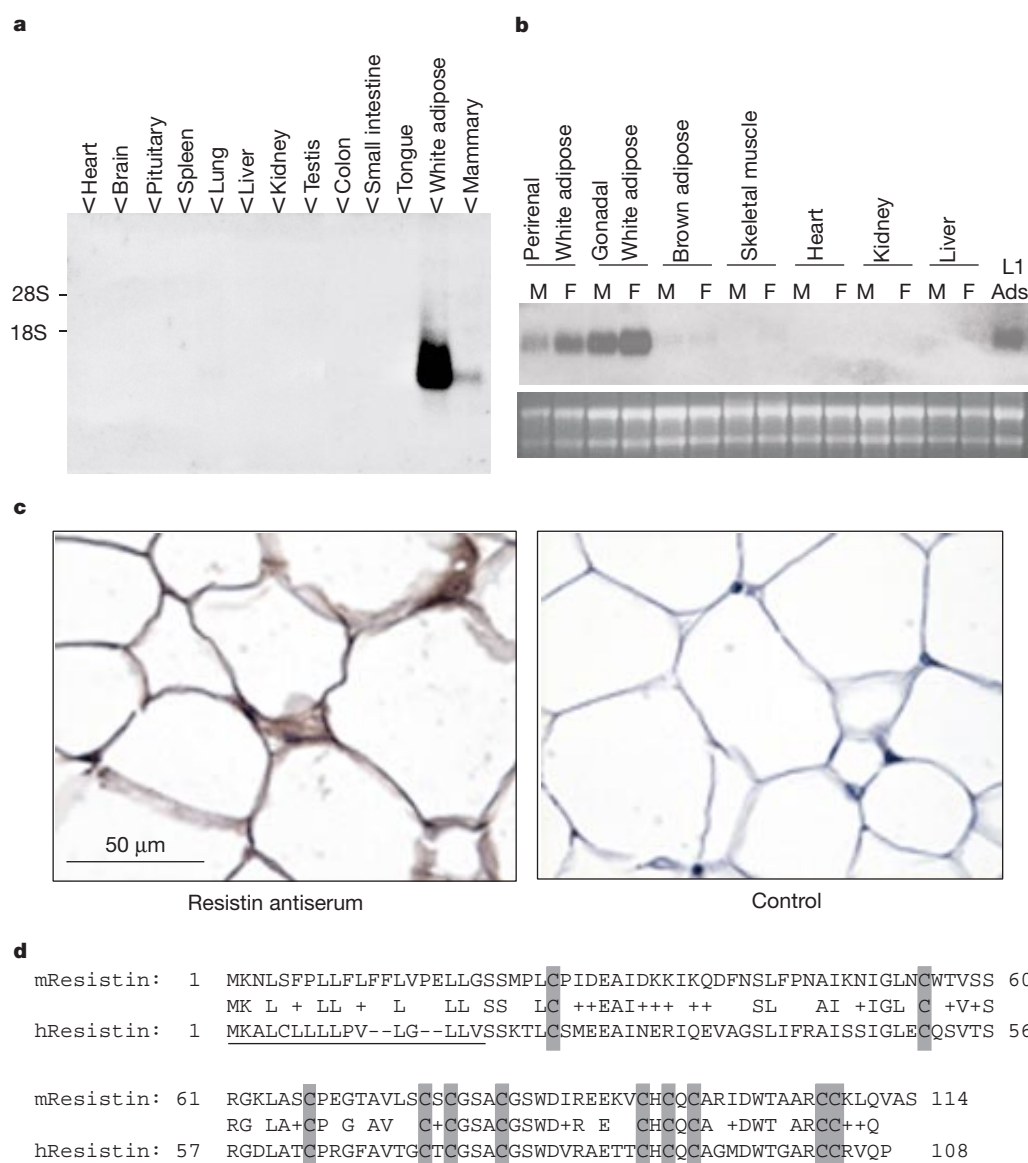


Figure 2 Resistin expression is adipocyte-specific. **a**, Tissue expression of resistin mRNA. **b**, Gender and depot effects on resistin expression. Tissues were pooled from three 10-week-old male (M) and female (F) mice. 28S and 18S RNA are shown. **c**, Expression of resistin protein in white adipose tissue. Resistin staining is brown and counterstain is blue.

d, Putative human resistin. The sequence of the protein encoded by a human cDNA that is highly homologous to resistin is shown. Putative signal sequence is underlined, and conserved cysteine residues are shaded.

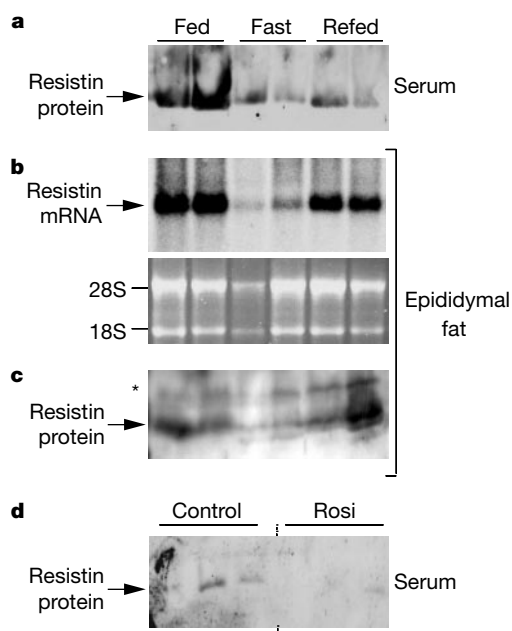


Figure 3 Resistin circulates in blood and is regulated by feeding and rosiglitazone. **a**, Resistin immunoblot of serum from C57Bl/6J mice with unrestricted feeding, fasted, or fasted and then refed. **b**, **c**, Adipocyte resistin gene expression (**b**) and protein (**c**) are reduced by fasting. Ribosomal RNA staining is shown as a control for loading of northern blot, and a nonspecific background band (asterisk) is nearly equal in all lanes of the immunoblot. **d**, Rosiglitazone treatment downregulates serum resistin levels.

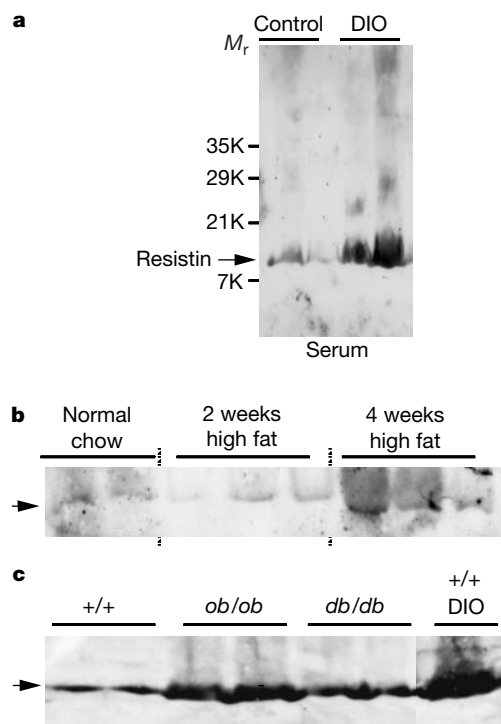


Figure 4 Resistin levels are elevated in obesity. **a**, Male AKR/J mice were fed a high-fat diet (DIO) or control chow from 4 to 12 weeks of age. Weight increased by 38% on the high-fat diet; serum insulin increased (2.1 ± 0.5 versus 5.8 ± 0.8 ng ml⁻¹). **b**, Time course. Male C57Bl/6J mice were fed normal chow (glucose 0.94 ± 0.04 mg ml⁻¹, insulin 0.47 ± 0.06 ng ml⁻¹) or a high-fat diet for 2 weeks (glucose 1.21 ± 0.07 mg ml⁻¹, insulin 2.6 ± 0.58 ng ml⁻¹) and 4 weeks (glucose 1.36 ± 0.04 mg ml⁻¹, insulin 3.6 ± 0.5 ng ml⁻¹). **c**, Resistin level in 10 μ l of serum from C57Bl/6J wild-type, *ob/ob*, *db/db* and diet-induced obesity (DIO) mice.

expression *in vivo* is specific to white adipose tissue, and resistin is found in the serum of normal mice. Resistin levels are increased in diet-induced obesity as well as in genetic models of obesity and insulin resistance. Resistin is therefore a candidate adipocyte-derived factor that contributes to insulin resistance *in vivo*. This hypothesis is supported by studies in adipocytes, where neutralization with resistin antiserum enhanced insulin-stimulated glucose uptake, and insulin action was blunted by recombinant resistin. Administration of resistin to mice impaired glucose tolerance without reducing insulin levels, and decreased sensitivity to the effects of insulin. Neutralization of resistin reduced the hyperglycaemia of obese, insulin-resistant mice, at least in part, by improving sensitivity to insulin. These data suggest that resistin is a unique hormone whose effects on glucose metabolism are antagonistic to those of insulin.

The effects of resistin administration and neutralization on glucose tolerance *in vivo* indicate that muscle, in addition to fat, may be a target for resistin; however, the molecular target of resistin is unknown. We speculate that resistin signals, by means of a receptor in insulin-responsive tissues, to modulate one or more steps in the insulin signalling pathway. Resistin may also have effects on tissues other than fat and muscle, such as liver and brain, and could have several functions. As with leptin²⁷, it seems probable that resistin did not evolve specifically to cause insulin resistance during times when food is plentiful. Rather, its physiological function may also be in the adaptive response to starvation. Additional work is required to understand better the biological function of resistin.

PPAR γ ligands represent an important breakthrough in the therapy of type 2 diabetes, and their ability to downregulate resistin suggests that resistin may be a link between obesity, diabetes and the

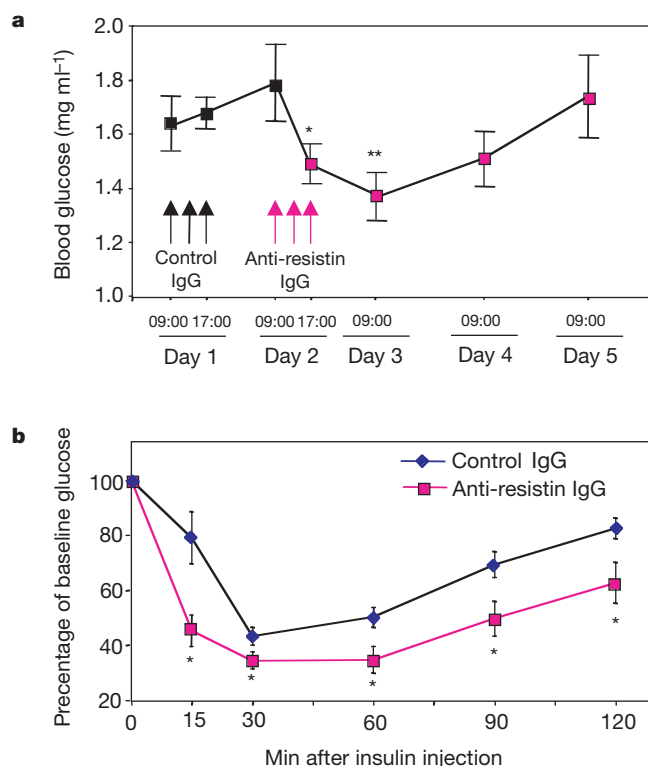


Figure 5 Neutralization of resistin improves hyperglycaemia and insulin resistance in mice. **a**, Glucose levels. C57Bl/6J mice were fed a high-fat diet from 8 to 16 weeks of age, then sequentially given three i.p. injections of 500 μ g control rabbit IgG or anti-resistin IgG (arrows). Data are mean \pm s.e.m. ($n = 4$). Asterisk, $P = 0.04$; double asterisk, $P = 0.02$ (paired *t*-test). **b**, Insulin tolerance testing. Mice received three i.p. injections of 1,000 μ g control rabbit IgG or anti-resistin IgG (see Methods). Data are mean \pm s.e.m. ($n = 6$) of the percentage of baseline glucose. Asterisk, $P = 0.0001$ (paired *t*-test).

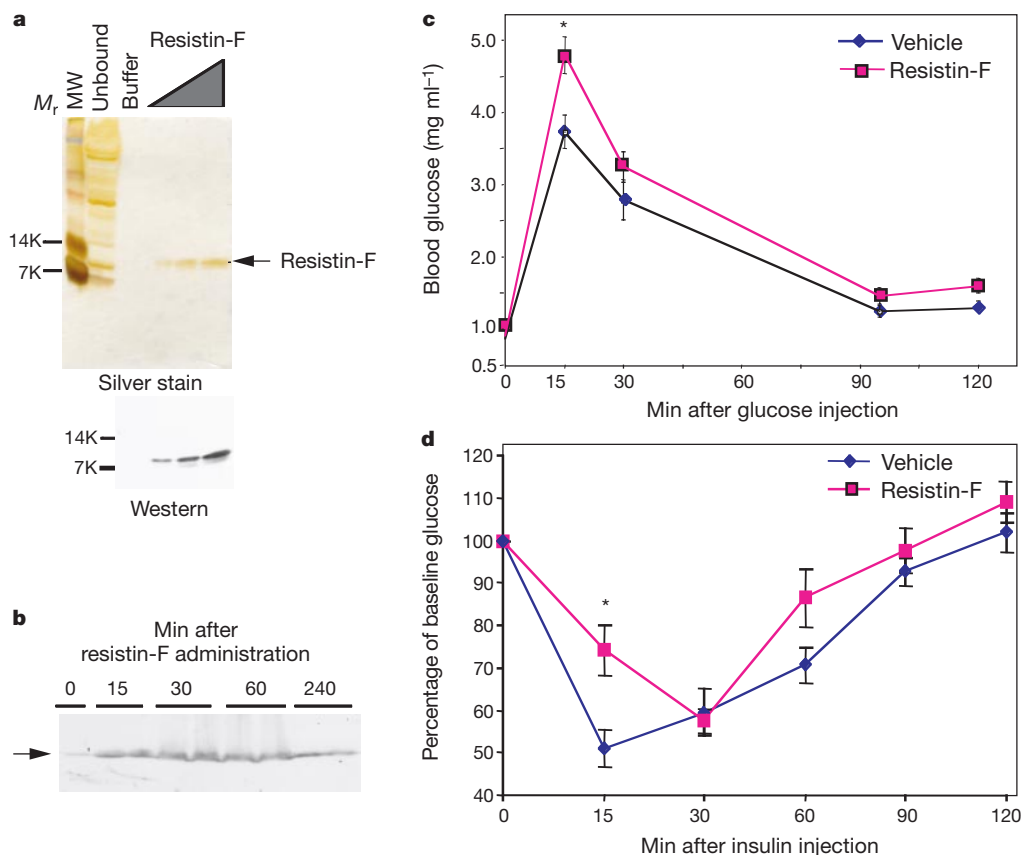


Figure 6 Resistin impairs glucose tolerance and insulin action in mice. **a**, Silver stain (top) and resistin immunoblot of 1, 2, and 4 μg purified resistin-F. MW, molecular mass markers. **b**, Serum levels of resistin at indicated times after a single dose of resistin-F (16.5 μg i.p.). **c**, Glucose tolerance. Resistin-F (16.5 μg) was administered i.p. to C57BL/

6J mice 14 h and 2 h before glucose administration. Data are mean \pm s.e.m. ($n = 15$ per group). Asterisk, $P = 0.004$ (unpaired t -test). **d**, Insulin tolerance. Mice were treated as in **c**, except that four doses of resistin-F were given. Data are mean \pm s.e.m. ($n = 9$ –10 per group) of the percentage of baseline glucose. Asterisk, $P = 0.003$ (unpaired t -test).

mechanism of action of these anti-diabetic drugs. Potential complications of PPAR γ activation in tissues other than fat^{28,29} may conceivably be avoided by making resistin the target of anti-diabetic therapy if the regulation and properties of human resistin are similar to those of mouse resistin. Such therapies could include

reduction of serum resistin level, neutralization of the biological activity of circulating resistin, and/or antagonism of resistin action directed against the cellular receptor(s). □

Methods

Identification and cloning of resistin

We differentiated 3T3-L1 cells as described³⁰. Adipocytes were treated for 48 h with either dimethyl sulphoxide or rosiglitazone (1 μM in dimethyl sulphoxide). Complementary DNA was prepared from 1 μg of RNA using the Cap finder cDNA synthesis kit (Clontech Labs, Palo Alto, California). We used PCR-select (Clontech) to select for adipocyte genes expressed in the absence of rosiglitazone. The cDNAs were arrayed on 96-well plates and screened using labelled cDNA from adipocytes or from adipocytes treated with rosiglitazone. Northern analysis of 20 μg of total RNA was performed as described¹⁵.

Resistin antibody

Resistin was expressed in bacteria as a glutathione S-transferase fusion protein that was used to generate rabbit polyclonal antiserum.

Immunoblot analysis

We performed immunoblot assays on whole-cell extracts, medium or serum as described³¹, except that we applied samples to 15% SDS-PAGE in loading buffer containing 20% β -mercaptoethanol. We used rabbit resistin antiserum at a dilution of 1:500 or 1:1,000.

Immunohistochemistry

Epididymal fat from a CD1 mouse was fixed in 4% paraformaldehyde in PBS. We analysed paraffin-embedded sections using rabbit anti-resistin polyclonal antiserum (1:500) and goat antirabbit antibody (1:200). We counterstained slides with Gill's haematoxylin number 2 (Fisher Scientific, Fairlawn, New Jersey).

Expression and purification of resistin and resistin-F

Wild-type resistin and resistin-F (with C-terminal Flag epitope created using PCR) cDNAs were subcloned into expression vectors pCMX or pZac2.1 and transfected into 293T cells

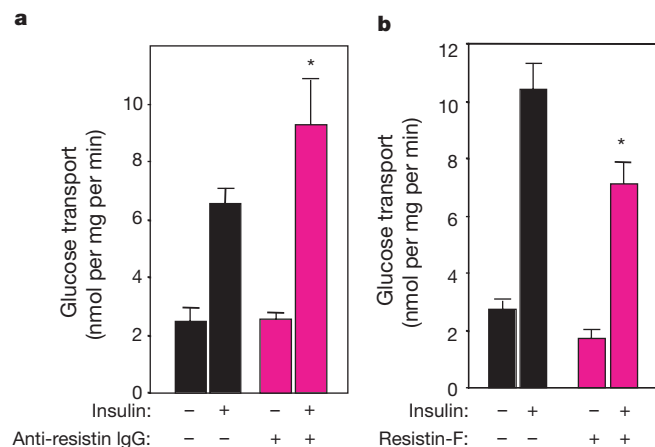


Figure 7 Resistin antagonizes insulin-stimulated glucose transport *in vitro*. **a**, 3T3-L1 adipocytes were stimulated with insulin (5 nM) after pretreatment with either control IgG or IgG purified from anti-resistin serum, and glucose uptake was measured. Mean \pm s.e.m. ($n = 3$) are shown for each data point. Asterisk, $P < 0.04$. **b**, 3T3-L1 adipocytes were treated with resistin-F (1–2 μM) or vehicle, with or without insulin (10 nM), and glucose uptake was measured. Mean \pm s.e.m. ($n = 6$) are shown for each data point. Asterisk, $P = 0.02$. The experiment shown is representative of three experiments.

using Fugene (Boehringer Mannheim). Resistin-F was batch-purified from conditioned media using Flag-Agarose (Sigma, St Louis, Missouri) and eluted with Flag peptide ($100 \mu\text{g ml}^{-1}$) in Tris-buffered saline. Pyrogen-free water was used in all steps of purification, and final endotoxin concentration of purified resistin-F and elution buffer control were determined to be less than 1 endotoxin unit per ml using the limulus amoebocyte lysate assay. We performed N-terminal sequencing of purified, secreted resistin-F by Edman reaction at the Wistar Protein Microchemistry Facility³².

In vivo experiments

Animal care and procedures were in accordance with guidelines and regulations of the institutional animal care and use committee of the University of Pennsylvania.

For fasting and rosiglitazone treatment, 2 weeks after acclimatization, 8-week-old male C57Bl/6J mice (Jackson Labs, Bar Harbor, ME) were housed ($n = 4$ per cage) under 12-h light/dark cycles (lights on at 06:00), ambient temperature 23°C , and allowed unrestricted access to chow and water. One group was maintained on chow, a second was fasted for 48 h, and a third was fasted for 48 h followed by unrestricted access to chow 48 h. We dissected epididymal fat for RNA and protein analysis. For rosiglitazone treatment experiments, female FVB/N mice, initially 5 weeks old, were treated with rosiglitazone ($0.012 \text{ mg per g diet}$; $\sim 3 \text{ mg per kg per day}$) for five weeks. Sera from these mice were provided by L. Chao and M. Reitman (Diabetes Branch, National Institutes of Diabetes, Digestive, and Kidney Diseases (NIDDK), Bethesda, Maryland).

For antibody neutralization, the effect of anti-resistin IgG on blood glucose was analysed in male C57Bl/6J mice fed on a high-fat diet from 8 to 16 weeks of age. After induction of diet-induced obesity, mice received three i.p. injections of sterile rabbit IgG ($500 \mu\text{g}$ in $50 \mu\text{l}$ 0.02 M potassium phosphate, 150 mM NaCl, $\text{pH } 7.2$) at 09:00, 12:00 and 15:00. The next day they received three i.p. injections of anti-resistin IgG ($500 \mu\text{g}$ in $50 \mu\text{l}$) at the above times. Tail bleeds were performed for glucose measurement at 09:00 and 17:00 on the day of treatment, and then daily for 3 d thereafter. The same protocol was used to evaluate the effect of anti-resistin IgG on insulin tolerance except that the dose of IgG was 1 mg . After the third dose of IgG, mice were fasted for 4 h before i.p. administration of insulin (0.75 U per kg Novolin R (Novo Nordisk, Princeton, New Jersey)).

To test the effects of resistin-F, serum resistin levels were analysed in normal chow-fed littermates at 0, 15, 30, 60 and 240 min after i.p. injection of resistin-F ($16.5 \mu\text{g}$ in Flag elution buffer). For glucose tolerance tests, male and female C57Bl/6J mice aged 5–8 weeks received resistin-F ($16.5 \mu\text{g}$) or vehicle i.p. before starting an overnight fast, and a second dose 12 h later. Two hours after the second dose, glucose (2 g kg^{-1} i.p.) was administered and glucose levels were measured in tail blood at indicated times. For insulin tolerance tests, mice received four doses of resistin-F or vehicle at 08:00, 14:00, 20:00 and 08:00 the next day, before a 4-h fast and administration of insulin (0.5 U per kg) and measurement of blood glucose.

Glucose transport assay

Glucose transport was assayed by measuring [^3H]2-deoxyglucose uptake as described³³. Twelve hours after serum starvation, cells were treated with insulin for 15 min at the indicated concentrations. Resistin rabbit polyclonal antibody (IgG) or IgG control was added at $9.4 \mu\text{g ml}^{-1}$ at the time of serum starvation. Resistin-F ($1\text{--}2 \mu\text{M}$) or Flag elution buffer was added at the time of serum starvation.

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- Nathan, D. M. Long-term complications of diabetes mellitus. *New Eng. J. Med.* **328**, 1676–1685 (1993).
- Taylor, S. I. Deconstructing type 2 diabetes. *Cell* **97**, 9–12 (1999).
- Kopelman, P. G. Obesity as a medical problem. *Nature* **404**, 635–643 (2000).
- Kahn, C. R., Vicent, D. & Doria, A. Genetics of non-insulin-dependent (type II) diabetes mellitus. *Annu. Rev. Med.* **47**, 509–531 (1996).
- Boden, G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **46**, 1–10 (1997).
- Hotamisligil, G. S. The role of TNF α and TNF receptors in obesity and insulin resistance. *J. Int. Med.* **245**, 621–625 (1999).
- Spiegelman, B. M. & Flier, J. S. Adipogenesis and obesity: rounding out the big picture. *Cell* **87**, 377–389 (1996).
- Mohamed-Ali, V., Pinkney, J. H. & Coppack, S. W. Adipose tissue as an endocrine and paracrine organ. *Int. J. Obes. Relat. Metab. Disord.* **22**, 1145–1158 (1998).
- Friedman, J. M. & Halaas, J. L. Leptin and the regulation of body weight in mammals. *Nature* **395**, 763–770 (1998).
- Shimomura, I., Hammer, R. E., Ikemoto, S., Brown, M. S. & Goldstein, J. L. Leptin reverses insulin

- resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* **401**, 73–76 (1999).
- Moller, D. E. Potential role of TNF α in the pathogenesis of insulin resistance and type 2 diabetes. *Trends Endocrinol. Metab.* **11**, 212–217 (2000).
- Henry, R. R. Thiazolidinediones. *Endocrinol. Metab. Clin. North Am.* **26**, 553–573 (1997).
- Lehmann, J. M. *et al.* An antidiabetic thiazolidinedione is a high affinity ligand for the nuclear peroxisome proliferator-activated receptor γ (PPAR γ). *J. Biol. Chem.* **270**, 12953–12956 (1995).
- Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I. & Spiegelman, B. M. mPPAR γ 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* **8**, 1224–1234 (1994).
- Chawla, A., Schwarz, E. J., Dimaculangan, D. D. & Lazar, M. A. Peroxisome proliferator-activated receptor γ (PPAR γ): Adipose predominant expression and induction early in adipocyte differentiation. *Endocrinology* **135**, 798–800 (1994).
- Barak, Y. *et al.* PPAR γ is required for placental, cardiac, and adipose tissue development. *Mol. Cell* **4**, 585–595 (1999).
- Rosen, E. D. *et al.* PPAR γ is required for the differentiation of adipose tissue *in vivo* and *in vitro*. *Mol. Cell* **4**, 611–617 (1999).
- Tontonoz, P., Hu, E. & Spiegelman, B. M. Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid-activated transcription factor. *Cell* **79**, 1147–1156 (1994).
- Willson, T. M., Brown, P. J., Sternbach, D. D. & Henke, B. R. The PPARs: from orphan receptors to drug discovery. *J. Med. Chem.* **2000**, 527–550 (2000).
- Mukherjee, R. *et al.* Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* **386**, 407–410 (1997).
- Barroso, I. *et al.* Dominant negative mutations in human PPAR γ associated with severe insulin resistance, diabetes mellitus, and hypertension. *Nature* **402**, 880–883 (1999).
- Kubota, N. *et al.* PPAR γ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* **4**, 597–609 (1999).
- Miles, P. D., Barak, Y., He, W., Evans, R. M. & Olefsky, J. M. Improved insulin-sensitivity in mice heterozygous for PPAR- γ deficiency. *J. Clin. Invest.* **105**, 287–292 (2000).
- Holcomb, I. N. *et al.* FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. *EMBO J.* **19**, 4046–4055 (2000).
- Steppan, C. M. *et al.* A family of tissue-specific resistin-like molecules. *Proc. Natl Acad. Sci. USA* (in the press).
- VanHeek, M. *et al.* Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J. Clin. Invest.* **99**, 385–390 (1997).
- Ahima, R. S. *et al.* Role of leptin in the neuroendocrine response to fasting. *Nature* **382**, 250–252 (1996).
- Seed, B. PPAR γ and colorectal carcinoma: conflicts in a nuclear family. *Nature Med.* **4**, 1004–1005 (1998).
- Tontonoz, P., Nagy, L., Alvarez, J. G., Thomazy, V. A. & Evans, R. M. PPAR γ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* **93**, 241–252 (1998).
- Shao, D. & Lazar, M. A. PPAR γ , C/EBP α , cell cycle status and the commitment to adipocyte differentiation. *J. Biol. Chem.* **272**, 21473–21478 (1997).
- Huang, E. Y. *et al.* Nuclear receptor corepressors partner with class II histone deacetylases in a Sin3-independent repression pathway. *Genes Dev.* **14**, 45–54 (2000).
- Speicher, D. W. & Reim, D. in *Current Protocols in Protein Science* (eds Coligan, J. E., Dunn, B. M., Ploegh, H. L., Speicher, D. W. & Wingfield, P. T.) 11.10.11–11.10.38 (John Wiley & Sons, New York, 1997).
- Hausdorff, S. F. *et al.* Identification of wortmannin-sensitive targets in 3T3-L1 adipocytes. *J. Biol. Chem.* **274**, 24677–24684 (1999).
- Nakai, K. & Horton, P. PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. *Trends Biochem. Sci.* **24**, 34–36 (1999).
- Nielsen, H., Engelbrecht, J., Brunak, S. & von Heijne, G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Engng* **10**, 1–6 (1997).

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