

LETTERS

Reactive oxygen species have a causal role in multiple forms of insulin resistance

Nicholas Houstis^{1,2,3}, Evan D. Rosen^{1,4} & Eric S. Lander^{1,2,3,5}

Insulin resistance is a cardinal feature of type 2 diabetes and is characteristic of a wide range of other clinical and experimental settings. Little is known about why insulin resistance occurs in so many contexts. Do the various insults that trigger insulin resistance act through a common mechanism? Or, as has been suggested¹, do they use distinct cellular pathways? Here we report a genomic analysis of two cellular models of insulin resistance, one induced by treatment with the cytokine tumour-necrosis factor- α and the other with the glucocorticoid dexamethasone. Gene expression analysis suggests that reactive oxygen species (ROS) levels are increased in both models, and we confirmed this through measures of cellular redox state. ROS have previously been proposed to be involved in insulin resistance, although evidence for a causal role has been scant. We tested this hypothesis in cell culture using six treatments designed to alter ROS levels, including two small molecules and four transgenes; all ameliorated insulin resistance to varying degrees. One of these treatments was tested in obese, insulin-resistant mice and was shown to improve insulin sensitivity and glucose homeostasis. Together, our findings suggest that increased ROS levels are an important trigger for insulin resistance in numerous settings.

Insulin resistance is a key feature of type 2 diabetes. It also occurs in such clinical settings as pregnancy, sepsis, cancer cachexia, obesity, starvation, acromegaly, burn trauma and metabolic syndrome, and in response to many experimental treatments *in vitro* and *in vivo*. We chose to study insulin resistance resulting from the treatment of 3T3-L1 adipocytes with either the inflammatory cytokine tumour-necrosis factor- α (mouse TNF) or the glucocorticoid dexamethasone. Both dexamethasone and TNF are well-validated experimental models of insulin resistance, and both have physiological relevance *in vivo*. Mice show impaired insulin sensitivity in response to TNF or dexamethasone treatment, and are protected from obesity-related insulin resistance by related physiological blockades (genetic ablation of TNF or the TNF receptor², or treatment with glucocorticoid antagonists³, respectively). Glucocorticoid treatment is also a frequent cause of insulin resistance in humans. Furthermore, elevated levels of TNF- α or glucocorticoids (or both) have been shown to be associated with insulin-resistant states such as obesity^{4,5}, cancer cachexia⁶, sepsis⁷, burn trauma⁸, pregnancy^{9,10}, metabolic syndrome¹¹ and starvation¹².

Despite these similarities between TNF and dexamethasone, their cellular response pathways are quite distinct: TNF signals through a cell-surface cytokine receptor, whereas dexamethasone signals through a nuclear hormone receptor. Moreover, TNF has pro-inflammatory properties, whereas dexamethasone is a prototypical anti-inflammatory agent. We reasoned that a powerful approach to understanding the cellular basis of insulin resistance would be to compare the effects of these two very different but

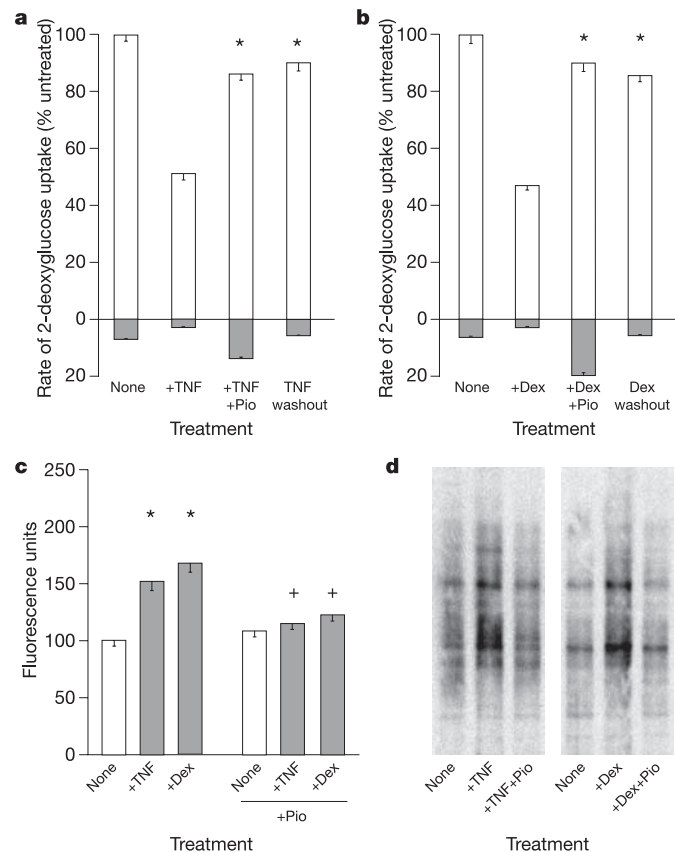


Figure 1 | Characterization of the insulin-resistant state. **a**, Rates of glucose transport in TNF-treated cells. Basal glucose transport (grey) and insulin-stimulated glucose transport (white) are shown. Cells were untreated, treated with TNF alone, TNF plus pioglitazone (Pio), or TNF followed by medium (TNF washout). Basal rate refers to the rate of glucose transport in the absence of insulin. Insulin-stimulated rate was calculated as the rate of transport in the presence of insulin minus the basal rate. All values are normalized to the insulin-stimulated rate from untreated cells. Asterisk indicates a significant difference ($P < 0.05$, *t*-test) compared to TNF treatment alone. **b**, Rates of glucose transport in dexamethasone (Dex)-treated cells. Data are analogous to **a**. **c**, Measurement of cellular redox status, showing rates of dichlorofluorescein (DCF) oxidation. Asterisk, $P < 0.05$ versus untreated; plus sign, $P < 0.05$ versus TNF or dexamethasone alone (*t*-tests). Results in **a–c** are mean \pm s.e.m. **d**, Measurement of chronic oxidative stress. Immunoblots show total protein carbonylation. In the left panel, lanes 1–3 were loaded with an equal amount of protein from cells that were untreated, treated with TNF alone or treated with TNF plus pioglitazone. The right panel is analogous, with dexamethasone replacing TNF.

¹Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02141, USA. ²Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. ³Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA. ⁴Division of Endocrinology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts 02214, USA. ⁵Department of Systems Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.

physiologically relevant treatments, as any pathways fundamental to insulin resistance might be expected to show responses in both settings.

Cultured 3T3-L1 adipocytes exposed to TNF or dexamethasone become insulin resistant within several days, as assessed by the ability of insulin to stimulate glucose uptake^{13,14}. To maximize the physiological relevance of this model, we calibrated the treatment regimen so that (1) insulin-dependent glucose uptake was decreased by ~50%, a degree similar to that seen in the clinical setting, (2) the defect in insulin action was reversible by washing out the agent, and (3) the defect could be rescued by pioglitazone (Fig. 1a, b), a member of the thiazolidinedione (TZD) class of insulin-sensitizing drugs.

We analysed genome-wide gene expression in TNF-treated, dexamethasone-treated and untreated adipocytes. Messenger RNA was prepared and hybridized to Affymetrix arrays containing probe sets from 22,690 mouse genes, with experiments performed in triplicate. Of the 350 upregulated genes showing the most robust expression change in response to TNF and dexamethasone treatments, only 34 of these were common to both treatments (Supplementary Table 1).

We analysed these results in two ways. First, we inspected the overlapping genes by eye, and noted that a substantial fraction (18%) was clearly related to the biology of reactive oxygen species (ROS). ROS are the radical forms of oxygen that arise as by-products of mitochondrial respiration and enzymatic oxidases. ROS are capable of acting as signalling molecules, but also cause damage to cellular

proteins, lipids and nucleic acids. The ROS-related genes with expression changes in response to both dexamethasone and TNF are shown in Supplementary Table 1.

Second, we applied the objective approach of gene set enrichment analysis (GSEA)^{15,16}. Given a collection of gene sets and the ranked list of gene expression changes resulting from a physiological treatment, GSEA tests whether the members of each set are randomly distributed along the list or clustered near the extremes, with the latter indicating that the gene set is regulated by the physiological treatment. From a curated collection of 475 gene sets¹⁶, GSEA identified ROS-related genes as the highest-scoring set for both dexamethasone treatment and TNF treatment (Supplementary Table 1b). Moreover, this was the only high-scoring set in common between the two treatments.

These gene expression results raise the possibility that ROS may be a key feature in both models of insulin resistance. We thus sought to test directly whether ROS levels are altered by TNF and dexamethasone treatment, by measuring oxidation of the redox-sensitive dye dichlorofluorescein (DCF). The resulting signal was higher by 50% or 65%, respectively, in insulin-resistant cells produced by TNF or dexamethasone treatment (Fig. 1c). Moreover, we found that increases in ROS levels precede the onset of detectable insulin resistance, becoming evident midway through treatment. In addition, we found that protein carbonyl levels, a marker of cumulative oxidative stress, were elevated by 50% and 110%, respectively,

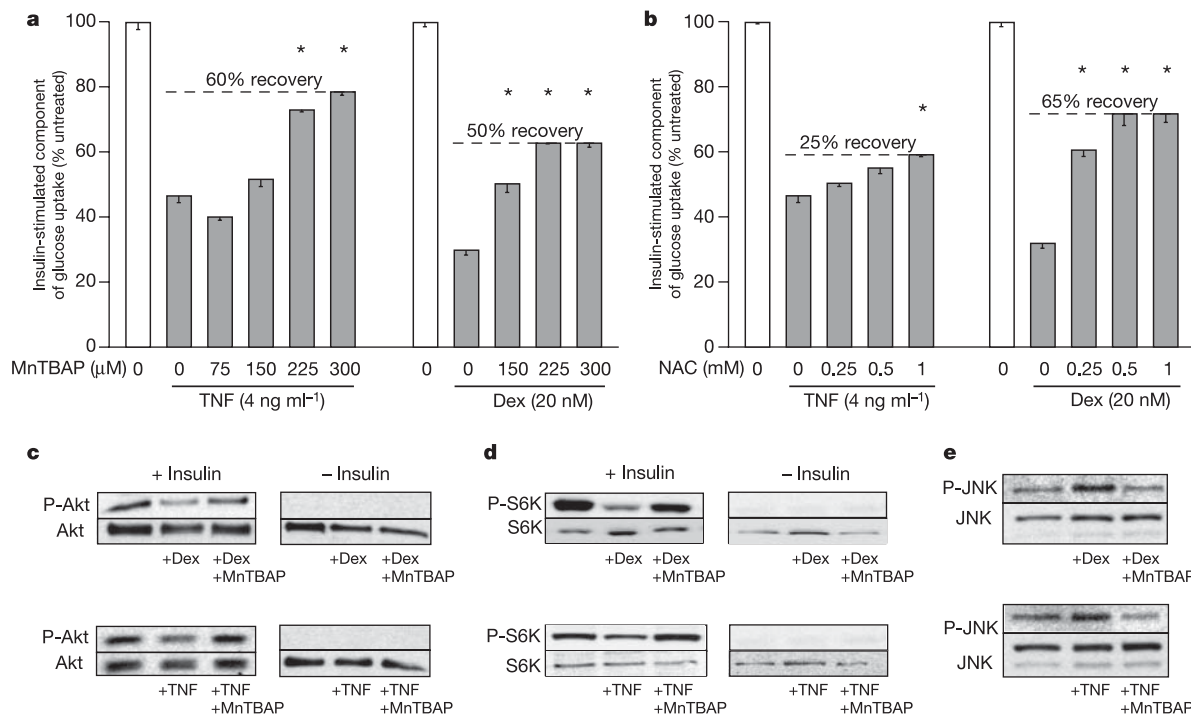


Figure 2 | Effect of antioxidants on insulin resistance. **a**, Partial restoration of TNF- or dexamethasone-induced insulin resistance by MnTBAP. Bars show insulin-dependent glucose transport. Results are mean \pm s.e.m. Asterisks indicate $P < 0.05$ versus TNF or dexamethasone (Dex) treatment alone (t -test). **b**, Partial restoration of TNF- or dexamethasone-induced insulin resistance by NAC. Data are analogous to **a**. **c–e**, Effect of 250 μ M MnTBAP on measures of insulin signal transduction in cells with dexamethasone-induced (top) or TNF-induced (bottom) insulin resistance. **c**, Immunoblots show phospho-Akt (P-Akt) levels on acute stimulation of cells with insulin (left) or in the absence of insulin (right). Total Akt protein levels are shown in the panel immediately below the phosphorylated counterpart. Signals were quantified by densitometry and normalized to total protein levels. The level of insulin-stimulated phospho-Akt decreases by 40% and 60%, respectively, in TNF- and dexamethasone-treated cells.

MnTBAP treatment recovers phospho-Akt levels to within 5% (TNF) and 30% (dexamethasone) of that found in untreated cells. **d**, Stimulation of p70S6K phosphorylation (P-S6K) by insulin was assayed, with data arranged and analysed as in **c**. The level of insulin-stimulated phospho-S6K decreases by 35% and 65%, respectively, in TNF- and dexamethasone-treated cells. MnTBAP treatment recovers phospho-S6K levels to within 25% (both TNF and dexamethasone) of that found in untreated cells. **e**, Immunoblot showing JNK phosphorylation (P-JNK) levels on treatment with dexamethasone, dexamethasone and MnTBAP, TNF, or TNF and MnTBAP. Total JNK levels are shown in the panel immediately below phospho-JNK. The level of phospho-JNK increases by 25% and 80%, respectively, in TNF- and dexamethasone-treated cells. MnTBAP treatment restores phospho-JNK to untreated levels.

in TNF- and dexamethasone-treated cells. When the TZD pioglitazone was co-administered with either TNF or dexamethasone, the level of DCF oxidation and the extent of protein carbonylation were both near that observed in untreated cells (Fig. 1c, d).

We next sought to test whether ROS have a causal role in insulin resistance by assessing whether a variety of treatments chosen specifically as suppressors of ROS levels could also act as insulin sensitizers. We began by evaluating two small antioxidant molecules, *N*-acetylcysteine (NAC) and manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP). NAC stimulates the formation of the endogenous reducing agent glutathione, which cells use to scavenge H₂O₂. MnTBAP has catalytic activities similar to the ROS-scavenging enzymes superoxide dismutase (SOD) and catalase; it protects mammalian cells from damage by H₂O₂ (ref. 17) and partially complements loss-of-function SOD mutations in bacteria and mice¹⁸. NAC and MnTBAP were applied to adipocytes concomitantly with TNF or dexamethasone. Both antioxidants showed dose-dependent suppression of insulin resistance induced by either treatment, preventing 25–65% of the defect in insulin-mediated glucose uptake (Fig. 2a, b); importantly, neither compound increased insulin action on its own (data not shown). Furthermore, MnTBAP largely prevented the increase in protein carbonylation (Supplementary Fig. 3).

We also examined the effect of MnTBAP on various parameters of insulin signalling. Treatment with TNF or dexamethasone decreases levels of insulin-stimulated serine phosphorylation on Akt and p70S6 kinases, whereas co-treatment with MnTBAP largely prevented this decrease (Fig. 2c, d). Increased ROS levels are known to stimulate threonine phosphorylation of JNK, a kinase previously linked to insulin resistance^{19,20}. TNF and dexamethasone treatment increased phosphorylation of JNK, whereas MnTBAP decreased it to nearly baseline levels (Fig. 2e).

We then explored the causal relationship between ROS and insulin

resistance by constructing four 3T3-L1 cell lines carrying transgenes encoding ROS-scavenging enzymes, including CuZnSOD, MnSOD, a form of catalase with its peroxisomal localization signal removed ('cytocalase'), and catalase targeted specifically to the mitochondrion ('mitocatalase') (see Methods). These transgenes were delivered by retroviral transduction, and expression was directed by an inducible promoter (Tet-on system) so as to minimize any effects of transgene expression on adipocyte differentiation. Transgene expression was induced before treatment with TNF or dexamethasone, resulting in a 3–5-fold increase in enzyme activity above endogenous levels (Supplementary Fig. 2). Mitocatalase and cytocalase were most potent, preventing up to 65% of the reduction in insulin-stimulated glucose uptake; CuZnSOD and MnSOD prevented 50% and 25%, respectively. Neither the transgene inducer (doxycycline) nor the inducible expression system itself had any effect on glucose transport (data not shown).

Together, our results using two small molecules and four transgenes indicate that diverse treatments chosen as suppressors of ROS can significantly reduce dexamethasone- and TNF-induced insulin resistance.

We next sought to extend these observations from cellular models to an *in vivo* model of insulin resistance, the leptin-deficient *ob/ob* mouse. These mice become extremely obese, developing significant insulin resistance and glucose intolerance by eight weeks of age. The mice are also known to exhibit markers of oxidative stress²¹. We tested whether chronic delivery of MnTBAP could improve glucose homeostasis. Male *ob/ob* mice received MnTBAP (at 2.5, 5 or 10 mg kg⁻¹ body weight), the TZD rosiglitazone (3 mg kg⁻¹ body weight) or vehicle alone. Treatments were administered daily, beginning at 8 weeks of age and continuing for a period of 12 weeks. The doses of MnTBAP are similar to or lower than doses shown to improve longevity in *Sod2* knockout mice¹⁸.

MnTBAP had no effect on the body weight of *ob/ob* mice over the

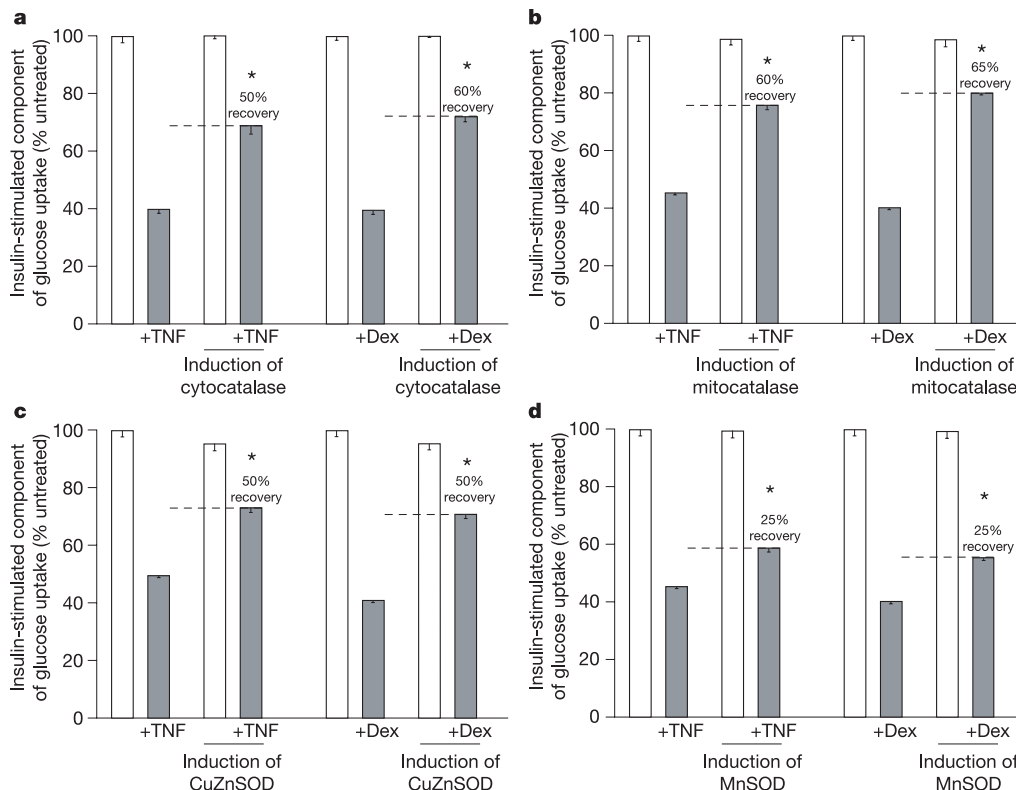


Figure 3 | Effect of transgenes on insulin resistance. a–d, Effect of cytocalase (a), mitocatalase (b), CuZnSOD (c) or MnSOD (d) transgene expression on TNF- and dexamethasone-induced insulin resistance. Bars show insulin-dependent rates of glucose transport in untreated cells with or

without transgene induction (white), or in TNF- or dexamethasone-treated cells with or without transgene induction (grey). Results are mean \pm s.e.m. Asterisks indicate $P < 0.05$ versus TNF or dexamethasone alone (*t*-test).

duration of the treatment period (Fig. 4a), but resulted in dose-dependent improvement in several measures of glucose homeostasis. For example, mice receiving the highest dose of MnTBAP showed nearly normal glucose levels in the fed state ($\sim 15\%$ above levels from control lean C57BL/6 mice in MnTBAP-treated versus $\sim 50\%$ in vehicle-treated mice) (Fig. 4b). MnTBAP improved glucose tolerance and insulin sensitivity (at 5 and 10 mg kg⁻¹), with a maximum effect comparable to that of animals treated with rosiglitazone (Fig. 4c, d). There was no significant effect on serum insulin levels in the fed or fasted state in MnTBAP-treated mice (data not shown).

In summary, these studies identify increased levels of ROS as a common feature of two models of insulin resistance, based on a genomic analysis that required no presupposition of which pathways might be involved. A causal role for ROS is shown by the observation that several pharmacological and genetic interventions designed to decrease ROS levels substantially prevent the development of insulin resistance. The treatment regimens tested in this study do not fully prevent the development of insulin resistance: this could either reflect that they do not restore ROS to normal levels, or could indicate that ROS levels act in parallel with other pathways. More detailed studies will be required.

ROS is one of many factors that have previously been suggested to

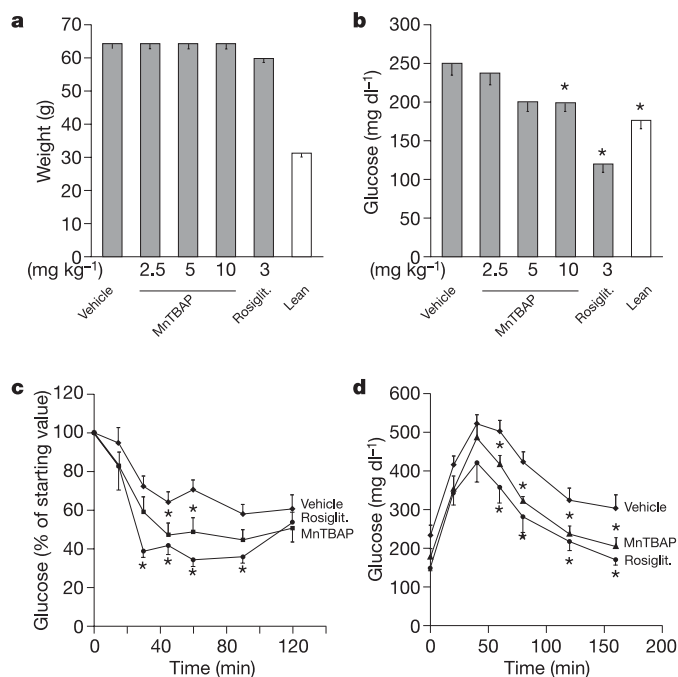


Figure 4 | Effects of chronic treatment with MnTBAP or rosiglitazone on obese mice. **a**, Effect on body weight. Bars show weights of animals after 12 weeks of treatment with daily subcutaneous injection of MnTBAP or rosiglitazone (Rosiglit.). Animals were treated with 2.5 mg kg⁻¹ ($n = 7$), 5 mg kg⁻¹ ($n = 8$) or 10 mg kg⁻¹ ($n = 8$) MnTBAP, 3 mg kg⁻¹ rosiglitazone ($n = 8$) or vehicle (phosphate-buffered saline) ($n = 8$). **b**, Effect on fed glucose levels. Bars indicate mean fed glucose levels averaged over 18 days during the last eight weeks of treatment. Glucose levels were determined at random times during the day and on random days during this period. **c**, Effect on insulin sensitivity. Insulin tolerance tests were performed on mice from each treatment group (vehicle, 5 mg kg⁻¹ MnTBAP or rosiglitazone). Lines indicate the time course of glucose levels after subcutaneous injection of human insulin (2 U kg⁻¹). **d**, Effect on glucose tolerance. Glucose tolerance tests were performed, with lines indicating the time course of glucose excursion following subcutaneous injection of glucose (1 g kg⁻¹) in vehicle-treated, 10 mg kg⁻¹ MnTBAP and rosiglitazone treatment groups. Results are mean \pm s.e.m. Asterisks indicate $P < 0.05$ versus vehicle-treated animals (t -test). Glucose and insulin tolerance test data using different dose levels of MnTBAP are provided in Supplementary Information.

have a possible role in insulin resistance, on the basis of two types of indirect evidence: (1) extensive association of markers of oxidative stress with obesity and diabetes^{22,23}, and (2) experiments showing that direct treatment of 3T3-L1 adipocytes with high doses of hydrogen peroxide²⁴ or with agents that induce ROS accumulation²⁵ can induce insulin resistance. However, such findings do not imply that ROS have a causal role in any physiological models of insulin resistance. This would require evidence that insulin resistance can be prevented to some degree by blocking the increase in ROS levels. To our knowledge, the only previous results that bear on this point are experiments showing that treatment of rodents and humans with the compound α -lipoic acid can partially improve insulin sensitivity. However, this evidence has been difficult to interpret because the action of α -lipoic acid is unclear: it can act either as an antioxidant or a pro-oxidant, and can directly stimulate insulin-independent glucose uptake²⁶. We also note a recent study that has suggested that a chemical inhibitor of NADPH oxidase can improve glucose homeostasis in obese KKAY mice²², although its effects on insulin resistance *per se* were not studied.

Our findings lead to a number of predictions. For example, one might predict that clinical conditions associated with insulin resistance will also show evidence of increased ROS levels. In fact, markers of oxidative stress are elevated in sepsis, burn injury, starvation, obesity, acromegaly and type 2 diabetes. In addition, conditions that increase ROS levels would be predicted to cause insulin resistance. In a literature review, we identified four human diseases with primary defects that affect ROS balance: familial amyotrophic lateral sclerosis, Friedrich's ataxia, ataxia telangiectasia and acatalasia. In the first three disorders, metabolic studies have demonstrated significant insulin resistance, although no explicit connection with ROS has previously been drawn. In the fourth case, insulin resistance *per se* has not been characterized, but patients have high rates of diabetes. (Detailed references concerning the above clinical conditions are provided in Supplementary Information.)

These findings raise issues that require further investigation. First, what downstream pathways translate elevated ROS levels into insulin resistance? ROS have been shown to induce various signalling pathways involving FoxO, MAPK, JAK/STAT, p53, phospholipase C, PI(3)K and other proteins. The particular pathway activated will depend on the magnitude of oxidative stress, the specific type of ROS, the cell type, the duration of exposure, and other factors. One attractive possibility is that ROS-induced insulin resistance is mediated by JNK. JNK is known to be activated by oxidative stress²⁷, and inhibition of JNK activity (through genetic knockout¹⁹ or an inhibitory peptide²⁰) improves insulin sensitivity in mice. Our data show that JNK is activated in response to both TNF and dexamethasone, and that this effect is reversed by MnTBAP. Second, what is the source of the ROS in insulin resistance? It is unclear whether increased ROS levels in various settings arise through a common mechanism or through different pathways. One candidate for a common mechanism for both TNF and dexamethasone might be the sphingolipid ceramide²⁸, which is increased in TNF- and dexamethasone-treated cells as well as diabetic muscle, and is capable of inducing mitochondrial ROS formation²⁹ and insulin resistance in 3T3-L1 cells³⁰. Third, what adaptive role might be served by a cellular mechanism that decreases insulin sensitivity in response to elevated ROS levels? Because an imbalance between substrate availability and oxidative capacity can lead to increased levels of ROS, cells may interpret elevated ROS levels as a signal to limit the input to the electron transport chain by decreasing glucose uptake. Fourth, are there inducers of insulin resistance that act on pathways downstream of ROS and thus are not associated with increased levels of ROS? Finally, through which tissue(s) do ROS mediate their effects on whole-body insulin resistance? Although our *in vitro* data show that adipocytes are one possible site of oxidant action, we note that the use of MnTBAP *in vivo* might affect other organs as well, such as muscle or liver.

Finally, our results suggest that antioxidant therapy might be a useful strategy in type 2 diabetes and other insulin-resistant states. Although some agents, such as vitamin E, have been clinically tested with ambiguous results, these have tended to be weak antioxidants with limited ROS-reducing effects. Newer, more effective antioxidant agents now under development for use in atherosclerosis and neurodegenerative disorders may prove worthy of investigation in this regard.

METHODS

A more complete description of the methods used is provided in Supplementary Information.

Cell culture. Early passage 3T3-L1 pre-adipocytes were cultured in Dulbecco's Modified Eagle Medium supplemented with Glutamax, 10% bovine calf serum, 100 U ml⁻¹ penicillin and 0.1 mg ml⁻¹ streptomycin. Differentiation, glucose uptake assays and western blots were performed under standard conditions (see Supplementary Information).

Induction of insulin resistance. Treatment with dexamethasone (20 nM) or recombinant mouse TNF (4 ng ml⁻¹) was initiated with mature adipocytes anywhere from day 8 to day 14 of differentiation. Media was changed daily for TNF treatment, for a total incubation time of 4 days. Dexamethasone media was changed every other day for a total of 8 days. For experiments in which transgene expression was induced, doxycycline was added to the cells on day 4 of differentiation and TNF or dexamethasone treatment was always initiated on day 8.

ROS measurement. For DCF assays, cells were washed twice in KRP buffer, incubated in pre-warmed KRP buffer containing 25 mM glucose and 5 μM CM-DCF, and placed at 37 °C. After 30 min, cells were washed once with KRP and fluorescence was immediately measured in a plate reader with an excitation/emission wavelength of 485/515 nm. DCF values were calculated after subtracting background fluorescence levels (measured under identical conditions but without DCF). For protein carbonylation assays, carbonyl levels were determined in 10 μg adipocyte protein lysate using a commercial kit (OxyBlot, Serologicals), according to the manufacturer's instructions.

Animals. Male *ob/ob* mice were purchased from Jackson Laboratories and randomly assigned to treatment group, so as to ensure that each group had an equal average weight. Drugs were resuspended in PBS and each mouse received daily 300-μl subcutaneous injections. Mice were weighed weekly.

Glucose and insulin tolerance tests. For the glucose tolerance test, mice were fasted for 12 h and then injected subcutaneously with glucose (1 g kg⁻¹ body weight). Blood samples were taken at regular time points (0–140 min), and blood glucose levels were determined with a portable glucose meter. For insulin tolerance tests, mice were fasted for 4 h and handled 30 min before performing the test. We then injected human regular insulin (2 U kg⁻¹ body weight) subcutaneously. Blood samples were taken at regular intervals (0–120 min) and blood glucose was measured as described above.

Received 23 November 2005; accepted 6 February 2006.

- Kroder, G. *et al.* Tumor necrosis factor- α - and hyperglycemia-induced insulin resistance. Evidence for different mechanisms and different effects on insulin signaling. *J. Clin. Invest.* **97**, 1471–1477 (1996).
- Uysal, K. T., Wiesbrock, S. M., Marino, M. W. & Hotamisligil, G. S. Protection from obesity-induced insulin resistance in mice lacking TNF- α function. *Nature* **389**, 610–614 (1997).
- Kusunoki, M., Cooney, G. J., Hara, T. & Storlien, L. H. Amelioration of high-fat feeding-induced insulin resistance in skeletal muscle with the antigluco-corticoid RU486. *Diabetes* **44**, 718–720 (1995).
- Bujalska, I. J., Kumar, S. & Stewart, P. M. Does central obesity reflect "Cushing's disease of the omentum"? *Lancet* **349**, 1210–1213 (1997).
- Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L. & Spiegelman, B. M. Increased adipose tissue expression of tumor necrosis factor- α in human obesity and insulin resistance. *J. Clin. Invest.* **95**, 2409–2415 (1995).
- McCall, J. L., Tuckey, J. A. & Parry, B. R. Serum tumour necrosis factor alpha and insulin resistance in gastrointestinal cancer. *Br. J. Surg.* **79**, 1361–1363 (1992).

- Stoner, H. B. *et al.* The effect of sepsis on the oxidation of carbohydrate and fat. *Br. J. Surg.* **70**, 32–35 (1983).
- Vaughan, G. M. *et al.* Cortisol and corticotrophin in burned patients. *J. Trauma* **22**, 263–273 (1982).
- Hornnes, P. J. On the decrease of glucose tolerance in pregnancy. A review. *Diabete Metab.* **11**, 310–315 (1985).
- Kirwan, J. P. *et al.* TNF- α is a predictor of insulin resistance in human pregnancy. *Diabetes* **51**, 2207–2213 (2002).
- Wang, M. The role of glucocorticoid action in the pathophysiology of the Metabolic Syndrome. *Nutr. Metab. (Lond)* **2**, 3 (2005).
- Balm, P. H. *Stress Physiology in Animals* (CRC Press, Boca Raton, 2000).
- Hotamisligil, G. S., Murray, D. L., Choy, L. N. & Spiegelman, B. M. Tumor necrosis factor α inhibits signaling from the insulin receptor. *Proc. Natl Acad. Sci. USA* **91**, 4854–4858 (1994).
- van Putten, J. P., Wieringa, T. & Krans, H. M. Corticosteroids as long-term regulators of the insulin effectiveness in mouse 3T3 adipocytes. *Diabetologia* **28**, 445–451 (1985).
- Mootha, V. K. *et al.* PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genet.* **34**, 267–273 (2003).
- Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl Acad. Sci. USA* **102**, 15545–15550 (2005).
- Day, B. J., Fridovich, I. & Crapo, J. D. Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. *Arch. Biochem. Biophys.* **347**, 256–262 (1997).
- Melov, S. *et al.* A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nature Genet.* **18**, 159–163 (1998).
- Hirosami, J. *et al.* A central role for JNK in obesity and insulin resistance. *Nature* **420**, 333–336 (2002).
- Kaneto, H. *et al.* Possible novel therapy for diabetes with cell-permeable JNK-inhibitory peptide. *Nature Med.* **10**, 1128–1132 (2004).
- Yang, S. *et al.* Mitochondrial adaptations to obesity-related oxidant stress. *Arch. Biochem. Biophys.* **378**, 259–268 (2000).
- Furukawa, S. *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. *J. Clin. Invest.* **114**, 1752–1761 (2004).
- Urakawa, H. *et al.* Oxidative stress is associated with adiposity and insulin resistance in men. *J. Clin. Endocrinol. Metab.* **88**, 4673–4676 (2003).
- Rudich, A. *et al.* Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes* **47**, 1562–1569 (1998).
- Lin, Y. *et al.* The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J. Biol. Chem.* **280**, 4617–4626 (2005).
- Moini, H., Tirosh, O., Park, Y. C., Cho, K. J. & Packer, L. R- α -lipoic acid action on cell redox status, the insulin receptor, and glucose uptake in 3T3-L1 adipocytes. *Arch. Biochem. Biophys.* **397**, 384–391 (2002).
- Kamata, H. *et al.* Reactive oxygen species promote TNF- α -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* **120**, 649–661 (2005).
- Summers, S. A. & Nelson, D. H. A role for sphingolipids in producing the common features of type 2 diabetes, metabolic syndrome X, and Cushing's syndrome. *Diabetes* **54**, 591–602 (2005).
- Di Paola, M., Cocco, T. & Lorusso, M. Ceramide interaction with the respiratory chain of heart mitochondria. *Biochemistry* **39**, 6660–6668 (2000).
- Summers, S. A., Garza, L. A., Zhou, H. & Birnbaum, M. J. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol. Cell. Biol.* **18**, 5457–5464 (1998).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank J. Zabolotny for assistance with signal transduction assays. We also thank D. Altshuler, O. Peroni and members of the Rosen and Lander laboratories for discussions. This work was supported in part by grants from the NIH (to E.D.R. and E.S.L.), the Hertz Foundation (N.H.) and the Broad Institute of MIT and Harvard.

Author Contributions E.D.R. and E.S.L. co-directed this work.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to E.D.R. (erosen@bidmc.harvard.edu) or E.S.L. (lander@broad.mit.edu).