Review

Why do anti-inflammatory therapies fail to improve insulin sensitivity?

Zhan-guo GAO, Jian-ping YE*

Antioxidant and Gene Regulation Lab, Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA 70808, USA

Chronic inflammation occurs in obese conditions in both humans and animals. It also contributes to the pathogenesis of type 2 diabetes (T2D) through insulin resistance, a status in which the body loses its ability to respond to insulin. Inflammation impairs insulin signaling through the functional inhibition of IRS-1 and PPAR γ . Insulin sensitizers (such as rosiglitazone and pioglitazone) inhibit inflammation while improving insulin sensitivity. Therefore, anti-inflammatory agents have been suggested as a treatment strategy for insulin resistance. This strategy has been tested in laboratory studies and clinical trials for more than 10 years; however, no significant progress has been made in any of the model systems. This status has led us to re-evaluate the biological significance of chronic inflammation in obesity. Recent studies have consistently asserted that obesity-associated inflammation helps to maintain insulin sensitivity. Inflammation stimulates local adipose tissue remodeling and promotes systemic energy expenditure. We propose that these beneficial activities of inflammation provide an underlying mechanism for the failure of anti-inflammatory therapy in the treatment of insulin resistance. Current literature will be reviewed in this article to present evidence that supports this viewpoint.

Keywords: inflammation; insulin resistance; type 2 diabetes; insulin sensitizer; obesity

Acta Pharmacologica Sinica (2012) 33: 182–188; doi: 10.1038/aps.2011.131; published online 31 Oct 2011

Introduction

For about two decades, it has been known that inflammation contributes to obesity-associated insulin resistance. Inflammatory cytokines (eg, TNF- α , IL-1, and IL-6) have been shown to induce insulin resistance in multiple organs (fat, muscle and liver). TNF- α elevation was found in adipose tissue of obese mice in 1993^[1]. That study provided the first evidence of the role of chronic inflammation during obesity and its association with insulin resistance in an animal model. Macrophages in adipose tissue are the major source of inflammatory cytokines in obesity^[2, 3]. Recent studies from multiple groups, including ours, consistently suggest that adipose tissue hypoxia is a root of chronic inflammation in obesity^[4]. Hypoxia is likely the result of a reduction in blood flow to adipose tissue, which is supported by some studies in humans and animals^[5–7].

In addition to adipose tissue hypoxia, metabolites of fatty acids and glucose, including diacylglyceride (DAG), ceramide, and reactive oxygen species, also contribute to the chronic inflammation in obesity. They activate the inflammatory response in several ways. They can directly interact with sig-

naling kinases (PKCs, JNKs, and IKKs) in cells^[8]; the lipids can also signal through cell membrane receptors for lipids, such as TLR4, CD36, or GPR^[8–13]. Fat or glucose oxygenation in the mitochondria can also generate reactive oxygen species (ROS), which can then induce activation of the inflammatory kinases (JNK and IKK) in the cytoplasm. The lipids also induce endoplasmic reticulum (ER) stress to activate JNK and IKK^[14, 15]. In obesity, these signaling pathways are activated as a result of the surplus calories and involved in the pathogenesis of chronic inflammation.

Chronic inflammation and insulin resistance

At the molecular level, inflammation induces insulin resistance by targeting IRS-1 and PPAR γ .

Inflammation and IRS-1 (insulin receptor substrate 1)

In cellular models of insulin resistance, the pro-inflammatory cytokine, TNF- α , is widely used to induce insulin resistance. The data from these cellular studies suggest that TNF- α is a major risk factor for insulin resistance in obesity and other chronic diseases^[1, 16, 17]. TNF- α inhibits insulin signaling by serine phosphorylation of IRS-1, which leads to the dissociation of IRS-1 from the insulin receptor and causes degradation of IRS-1 protein^[17–19]. In the insulin signaling pathway, IRS-1

* To whom correspondence should be addressed.

E-mail yejp@pbr.edu

Received 2011-08-01 Accepted 2011-09-06

undergoes tyrosine in response to insulin stimulation, which leads to activation of the insulin signaling pathway, downstream PI3K/Akt activation, and Glut4 translocation to the cell membrane for glucose uptake. TNF- α induces insulin resistance by IRS-1 serine phosphorylation through the activation of several serine kinases, including JNK^[20, 21], IKK^[22], ERK^[23–25], PKC^[26–28], Akt^[28, 29], GSK-3^[30–32], IRAK^[33], and mTOR^[34, 35]. In a recent study, we showed that IKK2 (IKK β) inhibits IRS-1 function through the activation of S6K, which directly phosphorylates IRS-1 at multiple sites (such as S312/307 and S270/265) in TNF- α -treated cells^[22, 36]. Serine phosphorylation induces IRS-1 degradation and serves as a negative feedback signal to impair insulin action^[35].

Inflammation inhibits PPAR γ function

The IKK β /NF- κ B (nuclear factor kappa B) pathway is a dominant inflammatory signaling pathway. The pathway has been under active investigation in the obesity field after IKK β was found to induce insulin resistance in obese mice^[37]. The serine kinase IKK has three major isoforms, including IKK α (IKK1), IKK β (IKK2), and IKK γ , which requires IKK β for NF- κ B activation^[38]. In obesity, IKK β is activated by several intracellular signals, such as ROS, ER stress, DAG, and ceramide. IKK β is also activated by extracellular stimuli, including TNF- α , IL-1, fatty acids^[11] and hypoxia^[39]. IKK β induces NF- κ B activation by phosphorylation of the Inhibitor of Kappa B alpha (I κ B α)^[40].

NF- κ B is a ubiquitous transcription factor that is formed by two subunits of the Rel family, which includes seven members, p65 (RelA), p50 (NF- κ B1), c-Rel, RelB, p100, p105, p52^[41]. These members form a homodimer or heterodimer that regulates gene transcription. In most cases, NF- κ B is a heterodimer of p65 and p50. P65 contains the transactivation domain and mediates the transcriptional activity of NF- κ B. P50 inhibits the transcriptional activity of p65^[42], and the NF- κ B activity is enhanced in p50 knockout mice^[43]. NF- κ B inhibits PPAR γ function through the competition for transcriptional coactivators or the exchange of corepressors with PPAR γ ^[44]. This process is responsible for inhibiting PPAR-target genes, such as CAP and IRS-2. Our study shows that IKK promotes the activity of HDAC3 in the nuclear corepressor complex. IKK induces nuclear translocation of HDAC3 from the cytoplasm. In the cytosol, HDAC3 associates with I κ B α , and the degradation of I κ B α promotes HDAC3 translocation into the nucleus. The PPAR γ inactivation leads to suppression of IRS-2 expression, a signaling molecule in insulin signaling pathways for Glut4 translocation.

Free fatty acids and insulin resistance

Elevated plasma free fatty acids (FFAs) induce insulin resistance in obese and diabetic subjects^[45]. It was known as early as 1983 that lipid infusion caused insulin resistance^[46, 47]. To examine the mechanism by which FFAs induced insulin resistance *in vivo*, rats were tested in a hyperinsulinemic-euglycemic clamp after a 5-h infusion of lipids/heparin, which raises plasma FFA concentrations^[47]. FFAs resulted in an approximate 35% reduction in insulin sensitivity, indicated by the

glucose infusion rate ($P < 0.05$ vs control), and a 25% reduction in glucose transport activity, as assessed by 2-[1,2-³H]deoxy-glucose uptake *in vivo* ($P < 0.05$ vs control). PKC θ is a major kinase involved in FFA-induced insulin resistance^[48]. According to the Randle glucose-fatty acid cycle, the preferential oxidation of free fatty acids over glucose plays a major role in the pathogenesis of insulin sensitivity^[49]. Local accumulation of fat metabolites, such as ceramides, diacylglycerol or acyl-CoA, inside skeletal muscle and liver may activate a serine kinase cascade, leading to defects in insulin signaling and glucose transport^[50].

Inflammation and energy metabolism

Inflammation is associated with increased energy expenditure in patients with chronic kidney disease^[51], cachexia^[52], inflammatory bowel disease^[53] and Crohn's disease^[54]. NF- κ B activity can promote energy expenditure, as supported by documents on energy expenditure in cachexia^[55, 56] and infection. However, the role of NF- κ B in energy expenditure was not tested in transgenic models. To this end, we have investigated energy metabolism in transgenic mice with elevated NF- κ B activity. The transcriptional activity of NF- κ B is enhanced either by over-expression of NF- κ B p65 in the fat tissue, or inactivation of NF- κ B p50 by global gene knockout^[57, 58]. In these two models, inflammatory cytokines (TNF- α and IL-6) were elevated in the blood, and energy expenditure was increased both during the day and at night^[57, 58]. Expression of TNF- α and IL-1 mRNA was increased in adipose tissue and macrophages. These cytokines are positively associated with energy expenditure in the body^[56]. In transgenic mice with deficiencies in these cytokines or their receptors, energy accumulation is enhanced and energy expenditure is reduced. This positive energy balance has been reported in transgenic mice deficient in TNF- α ^[59], IL-1^[60], or IL-6^[61].

The above literature suggests that energy accumulation induces chronic inflammation. Inflammation may promote energy expenditure in a feedback manner to counteract an energy surplus^[62]. Inflammation may act in the peripheral organs/tissues, as well as in the central nervous system, to regulate energy balance. In the peripheral tissues, inflammation induces fat mobilization and oxidation to promote energy expenditure. In the central nervous system, inflammation can inhibit food intake and activate neurons for energy expenditure, while inhibition of inflammation leads to fat accumulation^[62].

Anti-inflammation therapies for insulin resistance

In clinical trials, high-dose salicylate was used to inhibit inflammation by targeting IKK/NF- κ B^[37, 63–65]. Salicylate reduces blood glucose by inhibiting IKK/NF- κ B, as seen decades ago in patients with diabetes^[64–66]. More studies demonstrated that high-doses of aspirin (~7.0 g/d) improved multiple metabolic measures in patients with T2D, including substantial reductions in fasting and postprandial glucose, triglycerides and FFAs. These changes were associated with reduced hepatic glucose production and improvements in

insulin-stimulated glucose disposal, assessed during hyperinsulinemic-euglycemic clamping^[63–65, 67]. Aspirin inhibits the activity of multiple kinases induced by TNF- α , such as JNK, IKK, Akt, and mTOR. It may enhance insulin sensitivity by protecting the IRS proteins from serine phosphorylation^[68]. However, the therapeutic value of high-dose aspirin is limited by its side effects, including gastrointestinal irritation and high risk of bleeding.

Statins, a class of anti-inflammatory drugs, have been shown to downregulate the transcriptional activity of NF- κ B, AP-1, and HIF-1 α ^[65, 69], with coordinated reductions in the expression of prothrombotic and inflammatory cytokines. Randomized clinical trials have demonstrated that statins reduces CRP, multiple cytokines, and inflammatory markers in the body. Even with modest anti-inflammatory properties, statins do not appear to enhance insulin resistance or significantly improve glycemia^[70]. A recent review published in JAMA suggests that statin therapy is associated with excess risk for diabetes mellitus. The researchers analyzed five earlier trials, involving 32752 patients, to test the effect of the drug dose. Those getting intensive treatment were 12 percent more likely to have diabetes^[71], which translates into a 20 percent increase in developing diabetes in the high-dose statin users compared to those who do not take the drugs.

Glucocorticoids are the most effective anti-inflammatory drugs used to treat inflammatory diseases. Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs. In a clinical study, the effect of dexamethasone on insulin-stimulated glucose disposal was investigated with a double-blind, placebo-controlled, cross-over trial comparing insulin sensitivity (measured by the euglycemic hyperinsulinemic clamp) in young healthy males allocated the placebo or 1 mg dexamethasone twice daily for 6 d, each in random order. Six days of dexamethasone therapy was associated with a 30% decrease in insulin sensitivity^[72, 73]. This indicates that strong inhibition of inflammation may block the beneficial effects of inflammation on insulin sensitivity.

Interleukin-1 β induces inflammation in islets of patients with type 2 diabetes^[74]. The interleukin-1-receptor antagonist, a naturally occurring competitive inhibitor of interleukin-1^[75], protects human beta cells from glucose-induced functional impairment and apoptosis^[76]. The expression of the interleukin-1-receptor antagonist is reduced in pancreatic islets of patients with type 2 diabetes mellitus. High glucose induces the production of interleukin-1 β in human pancreatic beta cells, leading to impaired insulin secretion, decreased cell proliferation, and enhanced apoptosis. In this double-blind, parallel-group trial involving 70 patients with type 2 diabetes^[74], 34 patients were randomly assigned to receive 100 mg of anakinra (a recombinant human interleukin-1-receptor antagonist) subcutaneously once daily for 13 weeks. In the control group, 36 patients received placebo. All patients underwent an oral glucose-tolerance test. At the end of the study, the two study groups exhibited no difference in insulin resistance, insulin-regulated gene expression in skeletal muscle, serum adipokine levels, and the body-mass index. However, the therapy did

improve blood glucose levels. The authors conclude that the improvement is from enhanced pancreatic β -cell function. This study indicates that inhibition of IL-1 β improves glucose metabolism, independent of insulin sensitivity.

TNF- α expression is elevated in the adipose tissue of obese rodents and humans. In animal studies, administration of exogenous TNF- α induced insulin resistance, whereas neutralization of TNF- α improved insulin sensitivity. TNF- α knockout mice were used to examine the role of TNF- α in obesity-associated insulin resistance^[77]. The KO mice were compared with WT mice in lean and obese (induced by gold-thioglucose [GTG]-injection) conditions at 13, 19, and 28 weeks of age. In the lean condition, the KO mice exhibited a 14% reduction in body weight at 28 weeks of age. The epididymal fat pad was decreased by 25% in weight, relative to those of the wild-type littermate controls. Fasting glucose was reduced slightly by 10%, but the glucose response in an oral glucose tolerance test (OGTT) was not affected. In the obese condition, the body weight was identical between the KO and WT mice. Glucose levels were significantly increased in both groups during the OGTT. This indicates that the absence of TNF- α is not sufficient to protect mice from insulin resistance in obese conditions^[77]. Some animal studies^[78] and several clinical trials using TNF antagonism have thus far failed to improve insulin sensitivity^[79–83]. These facts suggest that there are many unknowns in the relationship of obesity-associated inflammation and insulin resistance.

The role of IL-6 in the pathogenesis of obesity and insulin resistance is controversial. IL-6 knockout (KO) mice were compared with WT littermate mice in lean or obese conditions. IL-6 KO mice displayed obesity, hepatosteatosis, liver inflammation and insulin resistance when compared with the lean condition on a standard chow diet^[84]. Overexpression of IL-6 was also used to test insulin resistance in mice. In the study, IL-6 overexpression was generated in skeletal muscle, and the IL-6 protein levels were increased in the circulation. The mice lost both body weight and body fat in response to IL-6 in this model, even though their food intake remained unchanged^[85]. These observations suggest that IL-6 increases energy expenditure. In the IL-6 mice, insulin levels were elevated, and hypoglycemia was observed^[85]. In another study, Sadagurski *et al* demonstrated that a high level of IL-6 in the circulation reduces obesity and improves metabolic homeostasis *in vivo*^[86].

The role of the anti-inflammatory cytokine IL-10 has been studied in the pathogenesis of obesity and insulin resistance^[87]. IL-10 is a critical cytokine of M2 (type 2) macrophages. A recent study has identified the roles of M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages in the regulation of insulin sensitivity^[88]. An increase in M2 macrophages and a decrease in M1 macrophages within the adipose tissue are associated with enhanced insulin sensitivity. In another study, the hematopoietic-cell-restricted deletion of IL-10 in mice was used to study the relationship between IL-10 and insulin resistance^[89]. The mice were assessed for insulin sensitivity in an insulin tolerance test in lean (chow diet) and obese (high fat diet) conditions. The results show that deletion of IL-10 from

the hematopoietic system does not have an effect on insulin resistance^[89]. Other studies suggest that IL-10 cannot improve insulin sensitivity in diet-induced obese mice or humans^[90, 91].

New potential drug candidates for insulin resistance

The antidiabetic drug thiazolidinedione (TZD) restores insulin action by activating PPAR γ , thus lowering the levels of FFAs in the blood. Activation of PPAR γ improves insulin sensitivity in rodents and humans through a combination of metabolic actions, including partitioning of lipid stores and regulating metabolic and inflammatory mediators, termed adipokines^[92]. However, TZD-based medicines for insulin sensitization have many side effects: troglitazone (Rezulin) was associated with massive hepatic necrosis; rosiglitazone (Avandia) and murglitazone, with increased cardiovascular events; and now, pioglitazone has been associated with bladder cancer^[93]. These adverse events suggest that the thiazolidinedione-based drugs may not be safe in the long-run. It is necessary to discover a new class of drug to treat insulin resistance.

Recent studies indicate that histone deacetylase (HDAC) inhibitors may be a new class of drug candidates for insulin sensitization. HDACs are key enzymes in regulating gene expression. Protein acetylation is one type of epigenetic regulation of gene expression. Acetylation is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetylation by HATs opens the chromatin structure to activate gene transcription, while histone deacetylases (HDACs) repress gene expression. HDACs are divided into three classes: class I HDACs (1, 2, 3, 8, 11), class II HDACs (4, 5, 6, 7, 9, 10)^[94] and class III HDACs (SIRT1-7)^[95]. Inhibition of histone deacetylase activity has been reported as a new approach to treat diabetes mellitus^[96-98]. In our study, supplementation of histone deacetylase inhibitors, butyrate or Trichostatin A, prevented high-fat diet-induced obesity and improved insulin sensitivity in mice. HDAC inhibition promoted energy expenditure, and reduced blood glucose and triglyceride levels in mice^[98]. HDAC inhibits insulin resistance on a molecular level by the following means: a) reducing the lipid toxicity^[44, 99-102]; b) reducing chronic systemic inflammation^[103-108]; c) promoting beta-cell development, proliferation, differentiation and function^[97]; and d) promoting energy expenditure^[98, 109]. Based on their multiple beneficial effects, HDAC inhibitors may represent a novel drug in the treatment of insulin resistance. However, clinical trials are needed to test this concept.

Conclusions

Type 2 diabetes is one of the major diseases associated with obesity. It is known that obesity promotes type 2 diabetes through insulin resistance, a state in which bodies lose their responsiveness to insulin. Many studies confirm that inflammation and free fatty acids (FFAs) are major pathogenic factors for insulin resistance in obese conditions. The most effective therapy for insulin resistance is to reduce both FFA and inflammation. Diminishing inflammation by anti-inflammatory drugs does not significantly improve insulin sensitiv-

ity in animal models or in clinical trials because inflammation is beneficial in regulating energy metabolism. Inhibiting this beneficial activity is likely to cause the failure of anti-inflammatory drugs in treating insulin resistance. Current literature consistently reports that fatty acids remain a therapeutic target in the treatment of insulin resistance. As an insulin sensitization-drug, TZD reduces both FFA and inflammation in the body. However, TZDs have many side effects such as obesity, heart attacks, and bladder cancer. HDAC inhibitors may be a new class of drug for treating insulin resistance by promoting energy expenditure and preventing obesity.

Acknowledgements

This work is partially supported by NIH grants DK068036 and DK085495 to Jian-ping YE and an NIH COBRE grant (2P20RR021945) and ADA grant (1-09-JF-17) to Zhan-guo GAO.

References

- 1 Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science* 1993; 259: 87-91.
- 2 Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003; 112: 1821-30.
- 3 Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; 112: 1796-808.
- 4 Ye J, Gao Z, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am J Physiol Endocrinol Metab* 2007; 293: E1118-28.
- 5 Larsen OA, Lassen NA, Quaade F. Blood flow through human adipose tissue determined with radioactive xenon. *Acta Physiol Scand* 1966; 66: 337-45.
- 6 Crandall DL, Goldstein BM, Huggins F, Cervoni P. Adipocyte blood flow: influence of age, anatomic location, and dietary manipulation. *Am J Physiol* 1984; 247: R46-51.
- 7 West DB, Prinz WA, Francendese AA, Greenwood MR. Adipocyte blood flow is decreased in obese Zucker rats. *Am J Physiol* 1987; 253: R228-33.
- 8 Brose N, Rosenmund C. Move over protein kinase C, you've got company: alternative cellular effectors of diacylglycerol and phorbol esters. *J Cell Sci* 2002; 115: 4399-411.
- 9 Costanzi S, Neumann S, Gershengorn MC. Seven transmembrane-spanning receptors for free fatty acids as therapeutic targets for diabetes mellitus: pharmacological, phylogenetic, and drug discovery aspects. *J Biol Chem* 2008; 283: 16269-73.
- 10 Aldhahi W, Hamdy O. Adipokines, inflammation, and the endothelium in diabetes. *Curr Diab Rep* 2003; 3: 293-8.
- 11 Lee JY, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, et al. Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. *J Biol Chem* 2003; 278: 37041-51.
- 12 Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F, Häring HU, et al. Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor- κ B. *J Biol Chem* 2004; 279: 23942-52.

- 13 Gao Z, Zhang X, Zuberi A, Hwang D, Quon MJ, Lefevre M, *et al*. Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. *Mol Endocrinol* 2004; 18: 2024–34.
- 14 Nakamura T, Furuhashi M, Li P, Cao H, Tuncman G, Sonenberg N, *et al*. Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. *Cell* 2010; 140: 338–48.
- 15 Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, *et al*. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004; 306: 457–61.
- 16 Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor- α in human obesity and insulin resistance. *J Clin Invest* 1995; 95: 2409–15.
- 17 Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- α - and obesity-induced insulin resistance. *Science* 1996; 271: 665–8.
- 18 Hotamisligil GS. The role of TNF α and TNF receptors in obesity and insulin resistance. *J Intern Med* 1999; 245: 621–5.
- 19 Peraldi P, Hotamisligil GS, Buurman WA, White MF, Spiegelman BM. Tumor necrosis factor (TNF)- α inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *J Biol Chem* 1996; 271: 13018–22.
- 20 Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 2000; 275: 9047–54.
- 21 Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001; 21: 27–41.
- 22 Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, *et al*. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J Biol Chem* 2002; 277: 48115–21.
- 23 De Fea K, Roth RA. Modulation of insulin receptor substrate-1 tyrosine phosphorylation and function by mitogen-activated protein kinase. *J Biol Chem* 1997; 272: 31400–6.
- 24 Engelman JA, Berg AH, Lewis RY, Lisanti MP, Scherer PE. Tumor necrosis factor α -mediated insulin resistance, but not dedifferentiation, is abrogated by MEK1/2 inhibitors in 3T3-L1 adipocytes. *Mol Endocrinol* 2000; 14: 1557–69.
- 25 Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, *et al*. Insulin/IGF-1 and TNF- α stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. *J Clin Invest* 2001; 107: 181–9.
- 26 De Fea K, Roth RA. Protein kinase C modulation of insulin receptor substrate-1 tyrosine phosphorylation requires serine 612. *Biochemistry*, 1997; 36: 12939–47.
- 27 Li Y, Soos TJ, Li X, Wu J, Degennaro M, Sun X, *et al*. Protein kinase C θ inhibits insulin signaling by phosphorylating IRS1 at Ser(1101). *J Biol Chem* 2004; 279: 45304–7.
- 28 Ravichandran LV, Esposito DL, Chen J, Quon MJ. Protein kinase C- ζ phosphorylates insulin receptor substrate-1 and impairs its ability to activate phosphatidylinositol 3-kinase in response to insulin. *J Biol Chem* 2001; 276: 3543–9.
- 29 Paz K, Liu YF, Shorer H, Hemi R, LeRoith D, Quan M, *et al*. Phosphorylation of insulin receptor substrate-1 (IRS-1) by protein kinase B positively regulates IRS-1 function. *J Biol Chem* 1999; 274: 28816–22.
- 30 Eldar-Finkelman H, Krebs EG. Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. *Proc Natl Acad Sci U S A* 1997; 94: 9660–4.
- 31 Ilouz R, Kowalsman N, Eisenstein M, Eldar-Finkelman H. Identification of novel glycogen synthase kinase-3 β substrate-interacting residues suggests a common mechanism for substrate recognition. *J Biol Chem* 2006; 281: 30621–30.
- 32 Liberman Z, Eldar-Finkelman H. Serine 332 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenuates insulin signaling. *J Biol Chem* 2005; 280: 4422–8.
- 33 Kim JA, Yeh DC, Ver M, Li Y, Carranza A, Conrads TP, *et al*. Phosphorylation of Ser24 in the pleckstrin homology domain of insulin receptor substrate-1 by Mouse Pelle-like kinase/interleukin-1 receptor-associated kinase: cross-talk between inflammatory signaling and insulin signaling that may contribute to insulin resistance. *J Biol Chem* 2005; 280: 23173–83.
- 34 Ozes ON, Akca H, Mayo LD, Gustin JA, Maehama T, Dixon JE, *et al*. A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. *Proc Natl Acad Sci U S A* 2001; 98: 4640–5.
- 35 Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM, *et al*. A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. *Mol Endocrinol* 2000; 14: 783–94.
- 36 Zhang J, Gao Z, Yin J, Quon MJ, Ye J. S6K directly phosphorylates IRS-1 on Ser-270 to promote insulin resistance in response to TNF-(α) signaling through IKK2. *J Biol Chem* 2008; 283: 35375–82.
- 37 Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, *et al*. IKK- β links inflammation to obesity-induced insulin resistance. *Nat Med* 2005; 11: 191–8.
- 38 Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF- κ B activity. *Annu Rev Immunol* 2000; 18: 621–63.
- 39 Cummins EP, Berra E, Comerford KM, Ginouves A, Fitzgerald KT, Seeballuck F, *et al*. Prolyl hydroxylase-1 negatively regulates I κ B kinase- β , giving insight into hypoxia-induced NF κ B activity. *Proc Natl Acad Sci U S A* 2006; 103: 18154–9.
- 40 Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. *Sci STKE* 2006; 2006: re13.
- 41 Baeuerle PA, Henkel T. Function and activation of NF- κ B in the immune system. *Annu Rev Immunol* 1994; 12: 141–79.
- 42 Schmitz ML, Baeuerle PA. The p50 subunit is responsible for the strong transcription activating potential of NF- κ B. *EMBO J* 1991; 10: 3805–17.
- 43 Bohuslav J, Kravchenko VV, Parry GC, Erlich JH, Gerondakis S, Mackman N, *et al*. Regulation of an essential innate immune response by the p50 subunit of NF- κ B. *J Clin Invest* 1998; 102: 1645–52.
- 44 Gao Z, He Q, Peng B, Chiao PJ, Ye J. Regulation of nuclear translocation of HDAC3 by I κ B α is required for tumor necrosis factor inhibition of peroxisome proliferator-activated receptor γ function. *J Biol Chem* 2006; 281: 4540–7.
- 45 Boden G. Free fatty acids (FFA), a link between obesity and insulin resistance. *Front Biosci* 1998; 3: d169–75.
- 46 Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA. Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* 1983; 72: 1737–47.
- 47 Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, *et al*. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. *Diabetes* 1999; 48: 1270–4.
- 48 Schmitz-Peiffer C, Oakes ND, Browne CL, Kraegen EW, Biden TJ.

- Reversal of chronic alterations of skeletal muscle protein kinase C from fat-fed rats by BRL-49653. *Am J Physiol* 1997; 273: E915–21.
- 49 Delarue J, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care* 2007; 10: 142–8.
- 50 Thompson AL, Cooney GJ. Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance. *Diabetes* 2000; 49: 1761–5.
- 51 Utaka S, Avesani CM, Draibe SA, Kamimura MA, Andreoni S, Cuppari L. Inflammation is associated with increased energy expenditure in patients with chronic kidney disease. *Am J Clin Nutr* 2005; 82: 801–5.
- 52 Moldawer LL, Georgieff M, Lundholm K. Interleukin 1, tumour necrosis factor- α (cachectin) and the pathogenesis of cancer cachexia. *Clin Physiol* 1987; 7: 263–74.
- 53 Barot LR, Rombeau JL, Steinberg JJ, Crosby LO, Feurer ID, Mullen JL. Energy expenditure in patients with inflammatory bowel disease. *Arch Surg* 1981; 116: 460–2.
- 54 Chan AT, Fleming CR, O'Fallon WM, Huizenga KA. Estimated versus measured basal energy requirements in patients with Crohn's disease. *Gastroenterology* 1986; 91: 75–8.
- 55 Strasser F. Appraisal of current and experimental approaches to the treatment of cachexia. *Curr Opin Support Palliat Care* 2007; 1: 312–6.
- 56 Tisdale MJ. Biology of cachexia. *J Natl Cancer Inst* 1997; 89: 1763–73.
- 57 Tang T, Zhang J, Yin J, Staszkiwicz J, Gawronska-Kozak B, Jung DY, *et al*. Uncoupling of inflammation and insulin resistance by NF- κ B in transgenic mice through elevated energy expenditure. *J Biol Chem* 2010; 285: 4637–44.
- 58 Gao Z, Yin J, Zhang J, He Q, McGuinness OP, Ye J. Inactivation of NF- κ B p50 leads to insulin sensitization in liver through post-translational inhibition of p70S6K. *J Biol Chem* 2009; 284: 18368–76.
- 59 Pamir N, McMillen TS, Kaiyala KJ, Schwartz MW, LeBoeuf RC. Receptors for tumor necrosis factor- α play a protective role against obesity and alter adipose tissue macrophage status. *Endocrinology* 2009; 150: 4124–34.
- 60 Chida D, Osaka T, Hashimoto O, Iwakura Y. Combined interleukin-6 and interleukin-1 deficiency causes obesity in young mice. *Diabetes* 2006; 55: 971–7.
- 61 Wallenius V, Wallenius K, Ahrén B, Rudling M, Carlsten H, Dickson SL, *et al*. Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med* 2002; 8: 75–9.
- 62 Ye J, Keller JN. Regulation of energy metabolism by inflammation: a feedback response in obesity and calorie restriction. *Aging (Albany NY)* 2010; 2: 361–8.
- 63 Kopp E, Ghosh S. Inhibition of NF- κ B by sodium salicylate and aspirin. *Science* 1994; 265: 956–9.
- 64 Pierce JW, Read MA, Ding H, Lusinskas FW, Collins T. Salicylates inhibit I κ B- α phosphorylation, endothelial-leukocyte adhesion molecule expression, and neutrophil transmigration. *J Immunol* 1996; 156: 3961–9.
- 65 Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; 116: 1793–801.
- 66 Williamson RT. On the treatment of glycosuria and diabetes mellitus with sodium salicylate. *Br Med J* 1901; 1: 760–2.
- 67 Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(κ)B kinase- β . *Nature* 1998; 396: 77–80.
- 68 Gao Z, Zuberi A, Quon MJ, Dong Z, Ye J. Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. *J Biol Chem* 2003; 278: 24944–50.
- 69 Dichtl W, Dulak J, Frick M, Alber HF, Schwarzacher SP, Ares MP, *et al*. HMG-CoA reductase inhibitors regulate inflammatory transcription factors in human endothelial and vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2003; 23: 58–63.
- 70 Chan DC, Watts GF, Barrett PH, Beilin LJ, Mori TA. Effect of atorvastatin and fish oil on plasma high-sensitivity C-reactive protein concentrations in individuals with visceral obesity. *Clin Chem* 2002; 48: 877–83.
- 71 Preiss D, Seshasai SR, Welsh P, Murphy SA, Ho JE, Waters DD, *et al*. Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy. *JAMA* 2011; 305: 2556–64.
- 72 Burén J, Liu HX, Jensen J, Eriksson JW. Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. *Eur J Endocrinol* 2002; 146: 419–29.
- 73 Perry CG, Spiers A, Cleland SJ, Lowe GD, Petrie JR, Connell JM. Glucocorticoids and insulin sensitivity: dissociation of insulin's metabolic and vascular actions. *J Clin Endocrinol Metab* 2003; 88: 6008–14.
- 74 Böni-Schnetzler M, Thorne J, Parnaud G, Marselli L, Ehses JA, Kerr-Conte J, *et al*. Increased interleukin (IL)-1 β messenger ribonucleic acid expression in beta-cells of individuals with type 2 diabetes and regulation of IL-1 β in human islets by glucose and autostimulation. *J Clin Endocrinol Metab* 2008; 93: 4065–74.
- 75 Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* 1996; 87: 2095–147.
- 76 Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, *et al*. Glucose-induced beta cell production of IL-1 β contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* 2002; 110: 851–60.
- 77 Ventre J, Doebber T, Wu M, MacNaul K, Stevens K, Pasparakis M, *et al*. Targeted disruption of the tumor necrosis factor- α gene: metabolic consequences in obese and nonobese mice. *Diabetes* 1997; 46: 1526–31.
- 78 Schreyer SA, Chua Jr SC, LeBoeuf RC. Obesity and diabetes in TNF- α receptor-deficient mice. *J Clin Invest* 1998; 102: 402–11.
- 79 Dominguez H, Storgaard H, Rask-Madsen C, Steffen Hermann T, Ihlemann N, Baunbjerg Nielsen D, *et al*. Metabolic and vascular effects of tumor necrosis factor- α blockade with etanercept in obese patients with type 2 diabetes. *J Vasc Res* 2005; 42: 517–25.
- 80 Lo J, Bernstein LE, Canavan B, Torriani M, Jackson MB, Ahima RS, *et al*. Effects of TNF- α neutralization on adipocytokines and skeletal muscle adiposity in the metabolic syndrome. *Am J Physiol Endocrinol Metab* 2007; 293: E102–9.
- 81 Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R. Effects of an engineered human anti-TNF- α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 1996; 45: 881–5.
- 82 Paquot N, Castillo MJ, Lefèbvre PJ, Scheen AJ. No increased insulin sensitivity after a single intravenous administration of a recombinant human tumor necrosis factor receptor: Fc fusion protein in obese insulin-resistant patients. *J Clin Endocrinol Metab* 2000; 85: 1316–9.
- 83 Rosenvinge A, Krogh-Madsen R, Baslund B, Pedersen BK. Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNF α therapy. *Scand J Rheumatol* 2007; 36: 91–6.
- 84 Matthews VB, Allen TL, Risis S, Chan MH, Henstridge DC, Watson N, *et al*. Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. *Diabetologia* 2010; 53: 2431–41.

- 85 Franckhauser S, Elias I, Rotter Sopasakis V, Ferré T, Nagaev I, Andersson CX, *et al*. Overexpression of IL6 leads to hyperinsulinaemia, liver inflammation and reduced body weight in mice. *Diabetologia* 2008; 51: 1306–16.
- 86 Sadagurski M, Norquay L, Farhang J, D'Aquino K, Copps K, White MF. Human IL6 enhances leptin action in mice. *Diabetologia* 2010; 53: 525–35.
- 87 Hong EG, Ko HJ, Cho YR, Kim HJ, Ma Z, Yu TY, *et al*. Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cytokine response in skeletal muscle. *Diabetes* 2009; 58: 2525–35.
- 88 Fujisaka S, Usui I, Bukhari A, Ikutani M, Oya T, Kanatani Y, *et al*. Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. *Diabetes* 2009; 58: 2574–82.
- 89 Kowalski GM, Nicholls HT, Risis S, Watson NK, Kanellakis P, Bruce CR, *et al*. Deficiency of haematopoietic-cell-derived IL-10 does not exacerbate high-fat-diet-induced inflammation or insulin resistance in mice. *Diabetologia* 2011; 54: 888–99.
- 90 den Boer MA, Voshol PJ, Schröder-van der Elst JP, Korshennikova E, Ouwens DM, Kuipers F, *et al*. Endogenous interleukin-10 protects against hepatic steatosis but does not improve insulin sensitivity during high-fat feeding in mice. *Endocrinology* 2006; 147: 4553–8.
- 91 Kohl A, Gögebakan O, Möhlig M, Osterhoff M, Isken F, Pfeiffer AF, *et al*. Increased interleukin-10 but unchanged insulin sensitivity after 4 weeks of (1,3)(1,6)-beta-glycan consumption in overweight humans. *Nutr Res* 2009; 29: 248–54.
- 92 Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem* 2008; 77: 289–312.
- 93 Ferrara A, Lewis JD, Quesenberry CP Jr, Peng T, Strom BL, Van Den Eeden SK, *et al*. Cohort study of pioglitazone and cancer incidence in patients with diabetes. *Diabetes Care* 2011; 34: 923–9.
- 94 Huang EY, Zhang J, Miska EA, Guenther MG, Kouzarides T, Lazar MA. Nuclear receptor corepressors partner with class II histone deacetylases in a Sin3-independent repression pathway. *Genes Dev* 2000; 14: 45–54.
- 95 Blander G, Guarente L. The Sir2 family of protein deacetylases. *Annu Rev Biochem* 2004; 73: 417–35.
- 96 Gilbert RE, Huang Q, Thai K, Advani SL, Lee K, Yuen DA, *et al*. Histone deacetylase inhibition attenuates diabetes-associated kidney growth: potential role for epigenetic modification of the epidermal growth factor receptor. *Kidney Int* 2011; 79: 1312–21.
- 97 Christensen DP, Dahlöf M, Lundh M, Rasmussen DN, Nielsen MD, Billestrup N, *et al*. Histone deacetylase (HDAC) inhibition as a novel treatment for diabetes mellitus. *Mol Med* 2011; 17: 378–390.
- 98 Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, *et al*. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009; 58: 1509–17.
- 99 Fajas L, Egler V, Reiter R, Hansen J, Kristiansen K, Debril MB, *et al*. The retinoblastoma-histone deacetylase 3 complex inhibits PPARgamma and adipocyte differentiation. *Dev Cell* 2002; 3: 903–10.
- 100 Guan HP, Ishizuka T, Chui PC, Lehrke M, Lazar MA. Corepressors selectively control the transcriptional activity of PPARgamma in adipocytes. *Genes Dev* 2005; 19: 453–61.
- 101 Miard S, Fajas L. Atypical transcriptional regulators and cofactors of PPARgamma. *Int J Obes (Lond)* 2005; 29: S10–2.
- 102 Zhang J, Henagan TM, Gao Z, Ye J. Inhibition of glyceroneogenesis by histone deacetylase 3 contributes to lipodystrophy in mice with adipose tissue inflammation. *Endocrinology* 2011; 152: 1829–38.
- 103 Rahman I. Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. *Biochem Pharmacol* 2002; 64: 935–42.
- 104 Adcock IM, Ito K, Barnes PJ. Histone deacetylation: an important mechanism in inflammatory lung diseases. *COPD* 2005; 2: 445–55.
- 105 Blanchard F, Chipoy C. Histone deacetylase inhibitors: new drugs for the treatment of inflammatory diseases? *Drug Discov Today* 2005; 10: 197–204.
- 106 Dinarello CA. Inhibitors of histone deacetylases as anti-inflammatory drugs. *Ernst Schering Res Found Workshop* 2006; 56: 45–60.
- 107 Zhang L, Fang H, Xu W. Strategies in developing promising histone deacetylase inhibitors. *Med Res Rev* 2010; 30: 585–602.
- 108 Shakespeare MR, Halili MA, Irvine KM, Fairlie DP, Sweet MJ. Histone deacetylases as regulators of inflammation and immunity. *Trends Immunol* 2011; 32: 335–43.
- 109 McGee SL, Hargreaves M. Histone modifications and exercise adaptations. *J Appl Physiol* 2011; 110: 258–63.