# New drug targets for type 2 diabetes and the metabolic syndrome

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**An insidious increase in features of the 'metabolic syndrome' — obesity, insulin resistance and dyslipidaemia — has conspired to produce a worldwide epidemic of type 2 insulin-resistant diabetes mellitus. Most current therapies for this disease were developed in the absence of defined molecular targets or an understanding of disease pathogenesis. Emerging knowledge of key pathogenic mechanisms, such as the impairment of glucose-stimulated insulin secretion and the role of 'lipotoxicity' as a probable cause of hepatic and muscle resistance to insulin's effects on glucose metabolism, has led to a host of new molecular drug targets. Several have been validated through genetic engineering in mice or the preliminary use of lead compounds and therapeutic agents in animals and humans.**

Type 2 insulin-resistant diabetes mellitus accounts for 90–95% of all diabetes. This heterogeneous disorder afflicts an estimated 6% of the adult population in Western society; its worldwide frequency is expected to continue to grow by 6% per annum, potentially reaching a total of 200–300 million cases in 2010 (refs 1, 2). The main force driving this increasing incidence is a staggering increase in obesity, the single most important contributor to the pathogenesis of diabetes.

It is now clear that aggressive control of hyperglycaemia in patients with type 2 diabetes can attenuate the development of chronic complications such as retinopathy and nephropathy[3]. At present, therapy for type 2 diabetes relies mainly on several approaches intended to reduce the hyperglycaemia itself: sulphonylureas (and related insulin secretagogues), which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production; peroxisome proliferator-activated receptor-γ (PPARγ) agonists (thiazolidinediones), which enhance insulin action; α-glucosidase inhibitors, which interfere with gut glucose absorption; and insulin itself, which suppresses glucose production and augments glucose utilization (Table 1). These therapies have limited efficacy, limited tolerability and significant mechanism-based side effects. Of particular concern is the tendency for most treatments to enhance weight gain. Several current approaches are also associated with episodes of hypoglycaemia, and few of the available therapies adequately address underlying defects such as obesity and/or insulin resistance. A problem particular to the sulphonylureas is that many patients who respond initially become refractory to treatment over time ('secondary failures'). Thus, newer approaches are desperately needed. Particular emphasis should be placed on finding and using mechanisms that are dependent on physiological responses (for example, glucose-mediated insulin secretagogues), and that result in weight loss (or lack of weight gain).

'Metabolic syndrome' encompasses type 2 diabetes (or prediabetes) and a common constellation of closely linked clinical features[4]. Characteristic factors include insulin resistance *per se*, obesity (in particular abdominal adiposity), hypertension, and a common form of dyslipidaemia (raised triglycerides and low high-density lipoprotein (HDL)–cholesterol with or without elevation of low-density lipoprotein (LDL)–cholesterol). Metabolic syndrome is associated with a markedly increased incidence of coronary, cerebral and peripheral artery disease. Thus, atherosclerotic cardiovascular disease (ASCVD) is responsible for 80% of diabetic mortality and more than 75% of all hospitalizations for diabetic complications. Indeed, type 2 diabetes now represents a coronary heart disease 'risk equivalent'; this means that the risk of myocardial infarction in patients with diabetes and no history of cardiac disease roughly equates to the risk in non-diabetic patients with known cardiac disease[5]. Put another way, for any abnormality in risk factors, diabetics have a two- to fourfold greater ASCVD risk than do people without diabetes. Thus, therapeutic approaches that not only lower glucose, but also specifically address diabetic dyslipidaemia and ASCVD complications are critically needed. In addition, current therapies do not directly address the other late-stage complications of diabetes (for example, neuropathy and retinopathy) that constitute a major disease burden (see the review in this issue by Brownlee, pages 813–820, for further discussion).

As therapeutic approaches for type 2 diabetes continue to evolve and improve, the goal of future treatment will be to intervene when very early clinical signs, such as impaired glucose tolerance and other aspects of metabolic syndrome, first manifest. The availability of drugs that affect underlying mechanisms may lead to a new therapeutic paradigm for the prevention of diabetes and its complications.

Current therapeutic approaches were largely developed in the absence of defined molecular targets or even a solid understanding of disease pathogenesis. Within the past few years, our understanding of biochemical pathways related to the

**Table 1 Current therapeutic agents for type 2 diabetes**

| Drug class | Molecular target | Site(s) of action | Adverse events |
|---|---|---|---|
| Insulin | Insulin receptor | Liver, muscle, fat | Hypoglycaemia, weight gain |
| Sulphonylureas (e.g. glibenclamide) plus nateglinide and repaglinide | SU receptor/ $K^+$ ATP channel | Pancreatic β-cell | Hypoglycaemia, weight gain |
| Metformin — biguanides | Unknown | Liver (muscle) | Gastrointestinal disturbances, lactic acidosis |
| Acarbose | α-glucosidase | Intestine | Gastrointestinal disturbances |
| Pioglitazone, rosiglitazone (thiazolidinediones) | PPARγ | Fat, muscle, liver | Weight gain, oedema, anaemia |

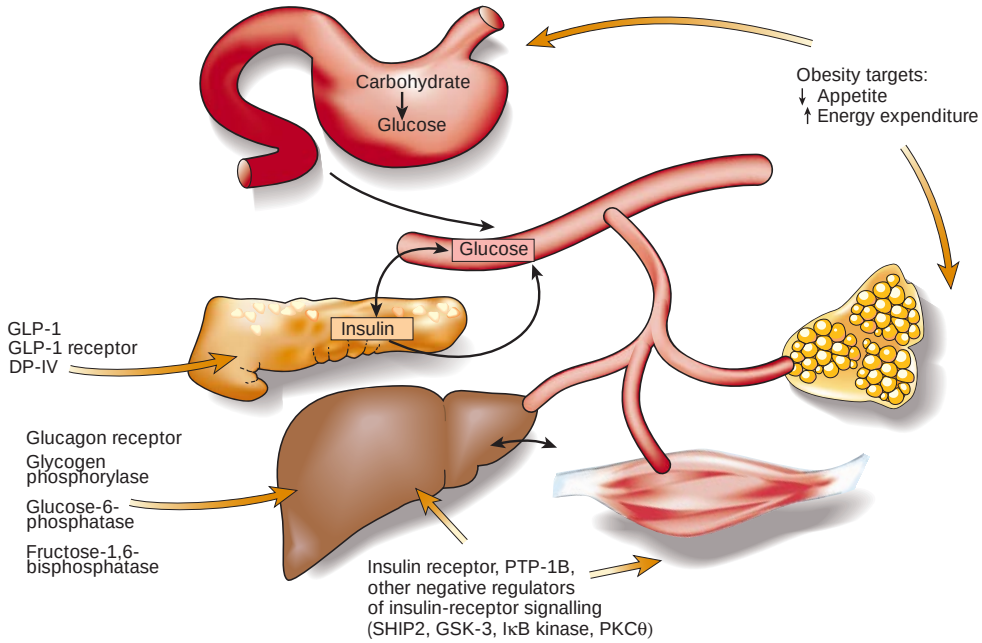


**Figure 1** A better understanding of defects involving several key organ systems has led to new drug targets for type 2 diabetes. The liver is largely responsible for unrestrained glucose production through increased rates of gluconeogenesis and glycogenolysis. Potential drug targets that modulate these processes include the glucagon receptor (antagonists), glycogen phosphorylase (inhibitors), and other rate-controlling enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase (inhibitors). Defective glucose-stimulated insulin secretion by pancreatic islet β-cells could be alleviated with recombinant glucagon-like peptide 1 (GLP-1) or agonists of the GLP-1 receptor. Alternatively, decreased GLP-1 clearance can be achieved with inhibition of dipeptidylpeptidase IV (DP-IV). To reduce insulin resistance, enhanced insulin action in liver and muscle (and fat) might be achieved with small-molecule activators of the insulin receptor or inhibitors of protein tyrosine phosphatase (PTP)-1B. Other potential drug targets in the insulin signalling pathway are discussed in the text. The development of anti-obesity agents that produce reduced appetite and/or increased energy expenditure will also lead to effective treatment (and prevention) of type 2 diabetes.

development of metabolic syndrome has expanded. There is an unprecedented range of molecular drug targets within these pathways. They have been identified on the basis of predicted roles in modulating one or more key aspects of the pathogenesis of diabetes and metabolic syndrome. Several mechanistic categories for new therapeutic approaches can be considered. First are approaches aimed at reducing excessive glucose production by the liver; second, mechanisms to augment glucose-stimulated insulin secretion; third, specific molecular targets in the insulin signalling pathway; and fourth, new approaches to obesity and altered lipid metabolism, which offer the prospect of net improvements in insulin action (or secretion) (Fig. 1).

## Reducing excessive hepatic glucose production

The liver has a critical role in regulating endogenous glucose production from *de novo* synthesis (gluconeogenesis) or the catabolism of glycogen (glycogenolysis). Increased rates of hepatic glucose production are largely responsible for the development of overt hyperglycaemia, in particular fasting hyperglycaemia, in patients with diabetes[6]. A relative decrease in insulin levels, or reduced hepatic responsiveness to insulin, can lead to increased output of glucose by the liver. Several drug targets in the liver offer new ways of attenuating excessive hepatic glucose production.

Glucagon is a well described hormone that contributes to hyperglycaemia through the induction of both gluconeogenic and glycogenolytic pathways[7–9]. The glucagon receptor, a seven-transmembrane domain G-protein-coupled receptor, is an obvious target for the development of small-molecule antagonists[10]. Neutralizing antibodies[11] and peptide-receptor antagonists[12] have both been shown to be effective antagonists *in vivo*. Several non-peptide glucagon-receptor antagonists have been reported so far[10]; although only one has shown hints of efficacy in early-stage human clinical trials[13].

Beyond the control of glucagon action, several enzymes that regulate rate-controlling steps in the gluconeogenic or glycogenolytic pathways are obvious molecular targets for therapeutic intervention (Fig. 2). An approach that seems to have advanced into early-stage clinical trials entails inhibition of hepatic glycogen phosphorylase[14], an enzyme that catalyses the release of monomeric glucose from stored glycogen. Despite some evidence that glycogenolysis may account for only a small fraction of liver glucose production in type 2 diabetes[15], an inhibitory molecule (a chloroindole carboxamide compound) that binds to a novel allosteric site (distinct from the enzyme active site) on glycogen phosphorylase has been highly effective in rodent models of diabetes[14]. The potential of such inhibitors to impair exercise-mediated catabolism of muscle glycogen is, however, a concern that merits further study.

Other hepatic enzyme targets that have received more limited attention include fructose-1,6-bisphosphatase and glucose-6-phosphatase[16,17]. Inhibition of the former would selectively block gluconeogenesis by disrupting the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. Inhibition of glucose-6-phosphatase would attenuate the final step in hepatic glucose production common to both gluconeogenic and glycogenolytic pathways. Although inhibition of hepatic glucose production remains attractive for further study, there are several potential liabilities inherent in this approach, including hypoglycaemia, accumulation of hepatic triglycerides and increased plasma lactate levels.

## Enhancing glucose-stimulated insulin secretion

A key component of the pathophysiology of type 2 diabetes involves a relatively selective defect in the ability of glucose to provoke secretion of insulin from pancreatic islet β-cells (see review in this issue by Mathis, Vence and Benoist, pages 792–798). This defect accounts for

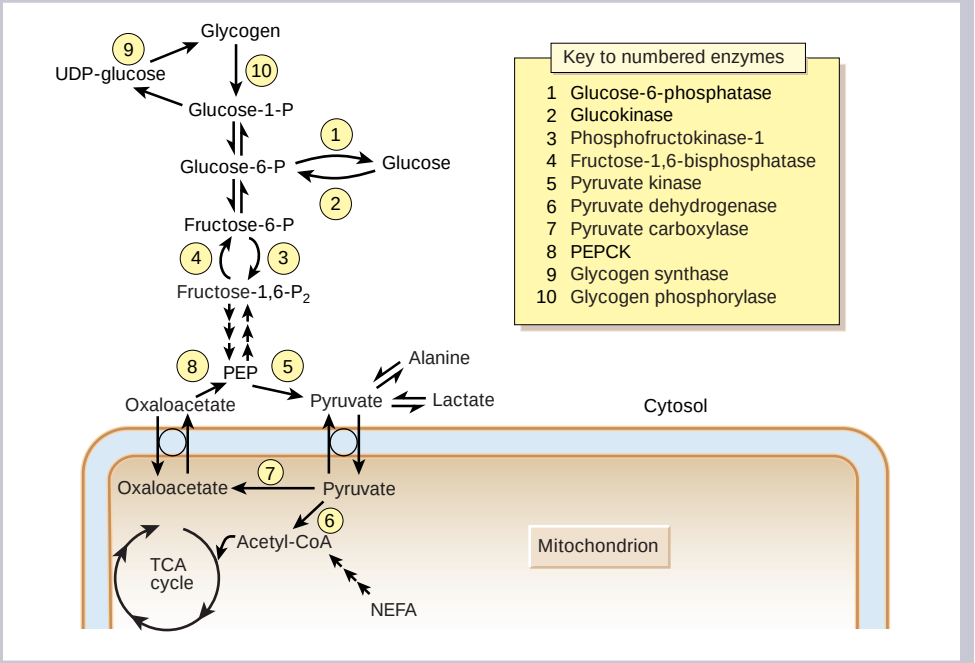


**Figure 2** Important pathways regulating glucose metabolism in the liver. Excessive hepatic glucose output occurs in diabetes through increases in glycogenolysis and/or gluconeogenesis. Inhibitors of glycogen phosphorylase inhibit glucose output by decreasing hepatic glycogen catabolism. Other relevant targets include fructose-1,6-bisphosphatase, which controls a rate-limiting step in gluconeogenesis, and glucose-6-phosphatase, which catalyses the final common step required for release of glucose from the liver. NEFA, non-essential fatty acids; PEP, phosphoenolpyruvate. [Adapted from ref. 77 with permission.]

the failure of the β-cell to compensate for increasing insulin resistance and for the ultimate development of overt hyperglycaemia. In addition, ample evidence shows that defective β-cell function can be an early predisposing factor[18]. Unlike sulphonylureas and related compounds, which stimulate insulin secretion in the absence of high glucose levels and work by blocking ATP-sensitive $K^+$ channels, more desirable alternative approaches would potentiate insulin secretion in a purely glucose-dependent fashion.

In this regard, two distinct gut-derived peptide hormones — glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) — act through their respective G-protein-coupled receptors on β-cells to potentiate glucose-stimulated insulin secretion[19]. Administration of either hormone to humans can potentiate insulin secretion, whereas selective gene disruptions of either the GLP-1 or GIP receptors produces a phenotype of impaired glucose-stimulated insulin secretion[19,20]. Additional mechanisms by which GLP-1 could have anti-diabetic effects include inhibition of gastric emptying, impairment of glucagon secretion and potential central anorexic effects[19]. Moreover, cell-based and animal studies suggest that GLP-1 has the potential to promote the growth of new islets and β-cell hyperplasia[19]. Administration of either exogenous GLP-1 or a potent GLP-1 agonist (exendin 4) derived from lizard venom has been shown to suppress energy intake in humans (reviewed in ref. 19), providing even stronger validation of GLP-1 as a therapeutic agent. A potential anti-obesity effect can thus be envisaged. In contrast, infusion of a GLP-1 antagonist (exendin 9-39 amide) impaired post-prandial glucose control[21].

Although both GLP-1 (and GIP) have strong potential as chronic therapies for diabetes, both are subject to rapid amino-terminal degradation ($t_{1/2}$ ~ 1 min) by dipeptidylpeptidase–IV (DP-IV, also known as CD26), a proline-specific serine dipeptidase. GLP-1 thus becomes inactivated by DP-IV *in vitro* ($k_{cat}/K_m \sim 1 \times 10^6\ M^{-1}\,s^{-1}$) to generate GLP-1[9–36] (Fig. 3). One approach to this problem could be the use of modified GLP-1 peptide agonists that are resistant to DP-IV (such as exendin 4). Importantly, specific DP-IV inhibitors have also been shown to increase circulating GLP-1 in both rodents and humans. Validation of DP-IV as a relevant drug target was bolstered by observations that DP-IV-null mice have increased circulating active GLP-1[7–36] along with enhanced insulin secretion and an otherwise healthy phenotype[22]. Moreover, early-stage clinical trials have provided 'proof-of-concept' for efficacy of DP-IV inhibition in humans with type 2 diabetes[23,24].

Although small-molecule agonists of the GLP-1 (and GIP) receptors would seem to provide a logical drug option, the existence of viable lead compounds has yet to be reported. The further development of GLP-1 analogues and DP-IV inhibitors is, however, likely to yield important new therapeutic approaches that might circumvent the liabilities of hypoglycaemia, weight gain and secondary failures associated with sulphonylurea use.

## Targeting the insulin signalling pathway

The role of peripheral and hepatic insulin resistance in the pathogenesis of diabetes is undisputed. As discussed by Saltiel and Kahn in an accompanying review (pages 799–806), insulin resistance can be due to multiple defects in signal transduction (such as impaired activation of insulin receptor-tyrosine kinase and reduced activation of insulin-stimulated phosphatidylinositol-3-OH kinase (PI(3)K)). A number of molecular targets are now being investigated as ways of enhancing insulin-mediated signal transduction (Table 2).

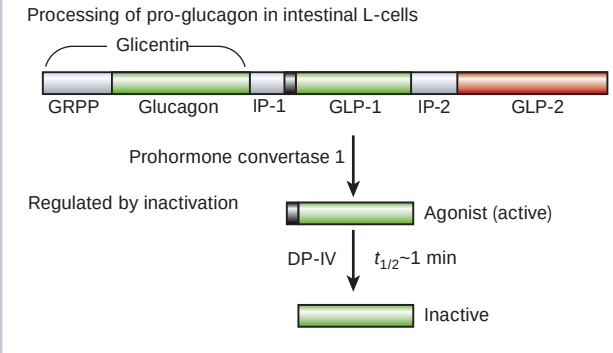


**Figure 3** Biosynthesis and regulation of glucagon-like peptide 1 (GLP-1). GLP-1 is a product of the pre-pro-glucagon gene. Pro-glucagon is cleaved by prohormone convertase 1 to generate active GLP-1[7-36], which is released from intestinal L-cells during nutrient ingestion. GLP-1 is rapidly hydrolysed *in vivo* ($t_{1/2}$ ~ 1 min) to produce an inactive product, GLP-1[9-36]. DP-IV, a proline-specific serine dipeptidase, is solely responsible for this inactivation. DP-IV inhibitors therefore represent an indirect therapeutic approach to stabilizing endogenous GLP-1.

Non-peptide small molecules that can activate the insulin receptor, or potentiate its activation by insulin, have proved elusive. But the recent discovery of a small-molecule natural-product derivative that mediates selective activation of the insulin receptor[25] is encouraging.

An alternative approach to targeting the insulin receptor itself would be to inhibit enzymes responsible for deactivation of the receptor or downstream targets in the signalling pathway (for example, IRS proteins). A number of specific protein tyrosine phosphatases (PTPs) have been identified as candidate targets[26]. Vanadium, peroxovanadium and their derivatives are non-selective PTP inhibitors. Nevertheless, demonstration of the insulin-sensitizing efficacy of vanadyl sulphate in humans suggests that one or more PTPs may be viable drug targets[27]. PTP-1B is an intracellular enzyme specifically implicated in the negative regulation of insulin signalling[26]. Recent results from genetic knockout of PTP-1B provide strong validation of this particular PTP as a potential target. The PTP-1B-null mice are healthy and have markedly enhanced sensitivity to insulin[28]. Surprisingly, they also showed substantial resistance to diet-induced obesity[28]. This observation is hard to reconcile with the fact that insulin serves as a major anabolic hormone to potentiate adipose accretion. Insulin action in the brain, however, may enhance satiety and mediate increased energy expenditure. Further validation of PTP-1B as a drug target has been provided by evidence of increased insulin action in insulin-resistant rats treated with a PTP-1B antisense oligonucleotide[29]. This treatment seems to have worked with injections of the antisense oligonucleotide once to twice weekly, and could be a viable approach for testing in humans.

Other putative negative regulators of insulin signalling (Table 2) have recently been implicated as independent drug targets. Glycogen synthase kinase-3 (GSK-3) has a clear role in opposing the effect of insulin, by inhibiting the activation of glycogen synthase and the subsequent accumulation of glycogen in muscle[30]. Recent results with potent and selective inhibitors suggest that reducing GSK-3 activity *in vivo* could indeed augment insulin action, and that this may occur at multiple steps[31]. This serine-threonine kinase may, however, have an important role in the regulation of cell proliferation and apoptosis through its function within the Wnt signalling pathway[30].

SH2-domain-containing inositol 5-phosphatase type 2 (SHIP2) may function to dephosphorylate key phospholipids (for example, phosphatidylinositol-3-phosphate; PtdIns(3)P) that are generated by insulin-mediated PI(3)K activation (see the review in this issue by Saltiel and Kahn, pages 799–806). This enzyme was implicated recently as a diabetes target, as heterozygous null mice have markedly enhanced sensitivity to insulin[32].

**Table 2 Potential drug targets in the insulin signalling pathway**

| Target | Validation | Potential mechanism(s) |
|---|---|---|
| Insulin receptor | Insulin, small-molecule activators/potentiators | Apparent direct activation of the receptor |
| PTP-1B | Efficacy of vanadium compounds; PTP-1B$^{-/-}$ (null) mice (insulin sensitive and obesity resistant); efficacy of PTP-1B antisense oligonucleotide | Mediates dephosphorylation of the insulin receptor (and its tyrosyl-phosphorylated substrates) |
| SHIP-2 | SHIP-2$^{+/-}$ mice (insulin sensitive) | Dephosphorylation of phosphoinositides (for example, products of PI(3)K) |
| GSK-3 | Efficacy of GSK-3 inhibitors in rodent models | Phosphorylation of glycogen synthase leading to inhibition of glycogen synthesis; potential negative regulation of other insulin signalling events |
| IκB kinase | Efficacy of high-dose salicylate (inhibits IκB kinase); IκB kinase$^{+/-}$ mice (insulin sensitive) | Serine–threonine phosphorylation of insulin signalling intermediates (for example, IRS proteins) |
| PKCθ | Activated in muscle in association with fatty-acid-induced insulin resistance | Negative regulation of insulin signalling; potential serine–threonine phosphorylation of IRS proteins |

Shoelson and colleagues recently revealed a new and intriguing potential diabetes drug target[33,34]. Two lines of evidence indicate an important role for IκB kinase (IKK) as a mediator of increased protein serine or threonine phosphorylation, which has the potential to downregulate insulin signalling. First, high-dose salicylate can produce increased insulin sensitivity in association with IKK inhibition, and second, heterozygous IKK-null mice have a phenotype of increased insulin sensitivity. As tumour necrosis factor-α (TNF-α), a potential mediator of obesity-associated insulin resistance[35], can activate the IKK complex, a specific role for IKK in TNFα-mediated insulin resistance may be implicated[33]. Similarly, protein kinase C-θ (PKCθ) could be an additional drug target, as increased muscle PKCθ activity has been observed in the context of fatty-acid-induced insulin resistance[36]. Further elucidation of key regulators in the insulin signalling pathway will no doubt lead to the discovery of additional drug targets for type 2 diabetes.

## Targeting obesity, lipid metabolism and 'lipotoxicity'

Given the critical role of obesity in the development of insulin resistance, and other features of the metabolic syndrome (see the reviews in this issue by Zimmet, Alberti and Shaw, pages 782–787, and Bell and Polonsky, 788–791), successful approaches to attenuating appetite and/or enhancing energy expenditure will prove of great benefit in preventing and treating type 2 diabetes. A wide range of drug targets for obesity *per se* is being actively investigated[37,38]. As an example of the potential benefits of modulating one such target, agonists of the melanocortin-4 receptor (MCR-4) offer the prospect of ameliorating obesity and type 2 diabetes. Thus, either an increase in the expression of a natural MCR-4 antagonist (Agouti) or knockout of the receptor itself produces a strong phenotype with multiple features of the metabolic syndrome[39,40]. An exciting new development[41] involves the realization that approaches designed to modulate central neuroendocrine systems that control energy metabolism, such as the melanocortin pathway, can have selective and beneficial effects on peripheral metabolism (for example, to alter insulin's effect on the liver *per se*). The prospect of a new approach to appetite reduction through central inhibition of fatty-acid synthase also offers an intriguing new avenue for drug research in this area[42].

Abnormalities of fatty-acid metabolism are increasingly recognized as key components of the pathogenesis of the metabolic syndrome and type 2 diabetes[43]. Fat-feeding and raised levels of circulating free fatty acids (FFAs) are clearly sufficient to induce peripheral and hepatic insulin resistance[44]. Accumulation of lipids inside muscle cells[45] and specific increases in muscle long-chain fatty-acyl-CoA content[44] have been implicated in causing insulin resistance. In addition, lipid accumulation within pancreatic islets has been proposed to impair insulin secretion[46]. A critical player in potentiating the promoting effect of hyperinsulinaemia on hepatic lipid accumulation is the anabolic transcription factor SREBP-1, which upregulates genes such as that for fatty-acid synthase[47]. These observations support a unified 'lipotoxicity' hypothesis, which states that metabolic syndrome and type 2 diabetes can be caused by the accumulation of triglycerides and long-chain fatty-acyl-CoA in liver and muscle (leading to a reduction in insulin-mediated metabolic activity) and in the islet (leading to impaired insulin secretion). Indeed, the weight-reducing hormone leptin may prevent diet-induced diabetes in rodents predominantly through reducing fat accumulation or 'steatosis' in these key tissues[48]. Several therapeutic targets discussed below, including AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), adipocyte-related complement protein 30 (Acrp30), PPARγ and PPARα, represent mechanisms that could be exploited to reverse or prevent obesity-related lipotoxicity.

### AMP-activated kinase and acetyl-CoA carboxylase

AMPK is activated in response to reduced cellular energy charge[49]. In turn, ACC, a key AMPK substrate, is inactivated in response to phosphorylation. As ACC catalyses the formation of malonyl-CoA, a

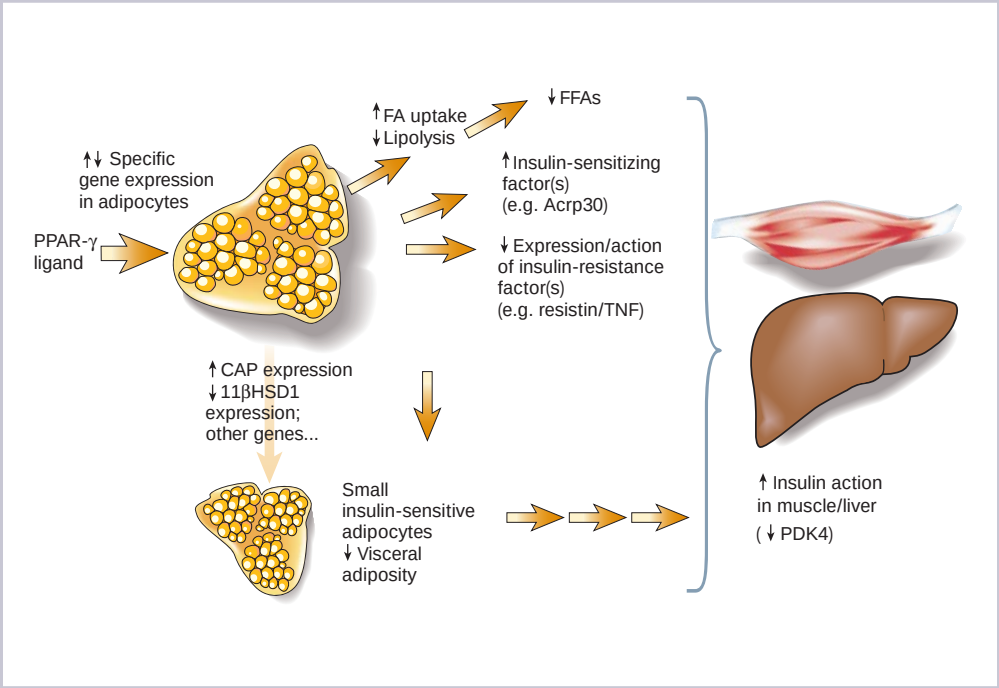


**Figure 4** Potential mechanisms of insulin sensitization by PPARγ ligands. The receptor PPARγ is predominantly expressed in adipose tissue. Ligand interactions with the receptor mediate specific changes in adipose gene expression. Altered expression of adipose genes such as fatty-acid transporter 1 may contribute to reduced production of free fatty acids (FFAs), which, in turn, is predicted to have insulin-sensitizing effects in muscle and liver. Changes in expression of other genes such as CAP or 11βHSD1 may contribute to locally increased insulin action in adipose tissue and/or reduced visceral adiposity. Altered expression of circulating factors including TNF-α, resistin and Acrp30 is also likely to indirectly mediate increased action of insulin in liver or muscle and glucose utilization; suppression of PDK4 activity in muscle is an example of one (probably indirect) effect.

potent inhibitor of fatty-acid oxidation and the first step in fatty-acid synthesis, AMPK activation and consequent ACC inactivation will result in reduced lipid synthesis and increased fat oxidation. AMPK activation also leads to reduced hepatic SREBP-1 and to suppressed expression of downstream lipogenic genes[49,50]. In addition, AMPK activation comprises a mechanism for exercise-mediated muscle glucose uptake[51,52]. Allosteric AMPK activation with an adenosine analogue, AICAR, has been shown to produce several beneficial metabolic effects, including inhibition of hepatic glucose output and increased muscle glucose uptake[49]. More recent results also suggest that the ability of the widely used drug metformin to inhibit hepatic glucose production and attenuate hepatic steatosis involves AMPK activation[50]. Taken together, the beneficial effects of AMPK activation by AICAR, and the probable role of AMPK activation as a contributor to the metabolic effects of metformin, provide a strong rationale for targeting AMP activation as a new therapeutic approach. ACC is also an exciting independent drug target, as mice deficient in one of two main ACC isoforms (ACC2) were characterized by increased fatty-acid oxidation with markedly reduced body weight and adiposity[53]. The extent to which ACC inhibition might protect against diabetes has not, however, been fully assessed.

### Adipocyte complement-related protein 30

Acrp30, or adiponectin, a secreted adipocyte-specific protein of $M_r$ 30,000, has recently been shown to produce beneficial metabolic effects in mice, including the ability to reduce glucose, triglycerides and FFAs[54–56]. This putative hormone may also induce tissue fatty-acid oxidation and reduce tissue steatosis in insulin-resistant animal models[54,56]. An additional acute effect in enhancing hepatic insulin action has also been suggested[55]. Therefore, either recombinant Acrp30 derivatives or small-molecule Acrp30-mimetic compounds could be envisaged as new therapeutic approaches.

## PPARs present multiple therapeutic targets

PPARs are ligand-activated transcription factors (members of the nuclear receptor family) which offer a promising therapeutic approach to the metabolic syndrome. The known beneficial effects of PPAR ligands are largely consistent with mechanisms that can ameliorate lipotoxicity. PPARγ is the predominant molecular target for insulin-sensitizing thiazolidinedione (TZD) drugs[57,58]. Surprisingly, the TZD drug class was discovered more than a decade before[59] this mechanism was deduced[60]. As TZDs suffer from only a modest net efficacy and several side effects, many investigators have been engaged in the search for improved PPARγ ligands as potential drugs. New compounds with markedly enhanced potency and selectivity for the receptor have recently been discovered[57].

A prevailing hypothesis for regulation of insulin sensitivity by PPARγ involves primary effects of PPARγ on gene transcription in adipose tissue (where it is most abundantly expressed), which ultimately lead to improved insulin action in muscle and liver (Fig. 4). Direct activation of PPARγ leads to the induction of adipocyte genes such as those for lipoprotein lipase and fatty-acid transporter 1, which in turn contribute to lowering triglyceride and FFA levels, respectively[61]. Similarly, suppression of TNF-α gene expression by PPARγ in adipose tissue has been reported[61]. As FFAs and TNF-α are both potential systemic mediators of insulin resistance, such effects are likely to contribute to the efficacy of PPARγ activation in increasing insulin sensitivity. As a consequence of reduced systemic lipid availability, muscle lipid levels can also be reduced[62].

Recent attempts to elucidate the genes regulated by PPARγ and involved in insulin sensitivity have shed light on several areas of interest for drug discovery research. In some of the examples discussed below, putative downstream targets of PPARγ (Fig. 4) were identified using PCR-differential messenger RNA display, DNA microarrays and related techniques[63–65]. Resistin is a novel protein secreted by adipose tissue with the apparent ability to antagonize insulin action[64]. Although adipose expression of resistin was initially reported to be suppressed by rosiglitazone, this finding has subsequently been questioned[66]. Muscle expression of the gene for pyruvate dehydrogenase kinase 4 (PDK4) was suppressed by *in vivo* treatment of rats with PPARγ agonists[65]. The net effect of inhibiting PDK4 should be to increase pyruvate dehydrogenase activity and to augment glucose utilization. In peripheral tissues, 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) catalyses the conversion of cortisone to the active glucocorticoid, cortisol. On the basis of the apparently insulin-sensitive phenotype of 11βHSD1-null mice, inhibition of this enzyme has been suggested as a potential drug target for metabolic syndrome[67]. Suppression of 11βHSD1 by PPARγ agonists in adipose tissue would seem to provide further validation for this target[68]. Similarly, *in vivo* activation of PPARγ in both

animals[56,69] and humans[70] has also recently been shown to increase circulating Acrp30 levels. Given the beneficial effects of recombinant Acrp30 described above, this finding represents an intriguing potential mechanism for insulin sensitization and is a further incentive to pursue Acrp30 as a target. PPARγ-mediated induction of the expression of an adaptor protein, *Cbl*-associated protein (CAP), also suggests an exciting mechanistic link between PPARγ and insulin sensitization of adipocytes *per se* (ref. 71, and see the review in this issue by Saltiel and Kahn, pages 799–806).

A closely related nuclear receptor, PPARα, is the molecular target for the fibrate class of lipid-modulating drugs[57]. Given the potential benefits of fibrate treatment for coronary disease, especially in patients with diabetes and the metabolic syndrome[72], and recent observations suggesting an independent insulin-sensitizing effect of PPARα agonists[73] (which could arise from reductions in muscle lipid content[74]), the incorporation of additional PPARα activity into compounds with PPARγ agonist activity has been proposed[75]. As adverse clinical effects of the TZD and fibrate drug classes are distinct, safe and effective insulin-sensitizing and lipid-altering compounds that engage both mechanisms of action as a single entity could possibly be developed.

By exploiting the fact that PPARs function like other nuclear receptors such as oestrogen receptors, it may also be assumed that compounds with tissue- or gene-specific effects could be identified. For the oestrogen receptor, binding of a partial agonist such as tamoxifen is associated with alternative receptor conformations, the potential for different profiles of receptor-associated co-factors, and selective biological responses versus classical agonists such as oestradiol[76]. Although specific genes that mediate the adverse and beneficial effects of PPARγ activation have yet to be fully characterized, an opportunity exists to determine *in vitro* profiles of full versus partial agonism and to test selected compounds *in vivo* to screen for those with an improved therapeutic index.

## Future directions

Our collective knowledge base of pathways and discrete proteins that contribute to distinctive pathophysiological traits underlying the metabolic syndrome and type 2 diabetes is expanding rapidly. This momentum is fuelled by the quantum leap in potential 'players' provided by the annotated human genome sequence databases and molecular techniques such as DNA microarrays and gene knockouts, and the identification of potential disease genes in humans and model species. The examples of recently discovered drug targets described above strongly suggest that this increase in newly identified components of disease susceptibility will yield an even wider array of potential approaches for therapeutic intervention. In addition to small-molecule modulators of 'classical' receptor or enzyme targets, research could identify additional protein therapeutics, such as GLP-1 analogues, and even more novel approaches, such as antisense oligonucleotide-based therapies.

Intensive study of the mechanisms of action of older drugs has provided further validation of several recently identified drug targets. Further efforts in this direction are likely to be fruitful. Given the multifactorial nature of the genetic and environmental factors that contribute to the genesis of metabolic syndrome and type 2 diabetes, it is probable that further efforts to characterize disease 'sub-phenotypes' and specific genetic markers will translate into more selective therapies tailor-made for distinct subgroups of patients or those at risk of developing disease. These individuals may be identified on the basis of specific genotypes or more specific clinical markers of distinct physiological derangements. □


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