Review

Nuclear Mechanisms of Insulin Resistance

Sona Kang,¹,² Linus T-Y. Tsai,¹ and Evan D. Rosen¹,*

Insulin resistance is a *sine qua non* of type 2 diabetes and is associated with many other clinical conditions. Decades of research into mechanisms underlying insulin resistance have mostly focused on problems in insulin signal transduction and other mitochondrial and cytosolic pathways. By contrast, relatively little attention has been focused on transcriptional and epigenetic contributors to insulin resistance, despite strong evidence that such nuclear mechanisms play a major role in the etiopathogenesis of this condition. In this review, we summarize the evidence for nuclear mechanisms of insulin resistance, focusing on three transcription factors with a major impact on insulin action in liver, muscle, and fat.

What Is Insulin Resistance and Why Is It Important?
Insulin resistance is the condition in which a cell, tissue, or organism fails to respond appropriately to a given dose of insulin. Insulin performs a wide variety of functions, and not all of these activities need to be dampened to make a diagnosis of insulin resistance; typically, insulin resistance refers to the metabolic actions of insulin and, specifically, to the ability of insulin to promote glucose uptake into tissues such as muscle and adipose and to repress glucose production in the liver. Insulin resistance is classically associated with type 2 diabetes, where it is a driver of hyperglycemia. Currently, type 2 diabetes affects 12–14% of US adults, and over half of all adults have prediabetes, a condition accompanied by significant insulin resistance [1].

Insulin resistance accompanies a wide range of pathological conditions, including obesity, lipodystrophy, sepsis, steroid use, growth hormone excess, polycystic ovarian disease, cancer, neurodegenerative disease, and even some physiological conditions, such as pregnancy. This can be modeled experimentally: investigators have developed numerous models of insulin resistance using a variety of chemical, drug, inflammatory, and nutritional challenges [2]. Thus arises a fundamental question: are there many independent paths to insulin resistance? Or do different perturbagens and clinical conditions converge on one or a few key pathways that are integral to any form of insulin resistance?

What Are the Current Theories about the Etiopathogenesis of Insulin Resistance?
Despite years of study, there is still great uncertainty concerning how cells and organisms become insulin resistant. Enormous effort has been expended delineating the signal transduction pathways activated by insulin, resulting in a fairly detailed map of the intermediates involved [2]. As predicted, mice lacking many of these signaling intermediates are profoundly insulin resistant, as are humans with similar loss-of-function mutations [3,4]. However, most insulin-resistant people do not harbor such mutations, so attention has focused on other causes of insulin resistance, including endoplasmic reticulum stress, inflammation, accumulation of toxic lipid intermediates (such as diacylglycerol and acylcarnitines), and reactive oxygen species (ROS) [5–8]. Data supporting the contribution of these non-mutually exclusive pathways to

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insulin resistance are strong, but mechanisms by which they ultimately affect insulin action are still unclear. Many investigators ultimately conclude that altered insulin signaling must underlie the final manifestation of the disease.

There are, however, suggestions that dysregulated insulin signaling per se does not lie at the heart of insulin resistance. First, experimental inducers of insulin resistance tend to provoke their effects over a time course inconsistent with alterations in signaling. For example, tumor necrosis factor (TNF) reduces insulin sensitivity in cultured adipocytes only after several days [9]. The received wisdom on TNF action, however, is that it induces inhibitory phosphorylation events on key components of the insulin signaling cascade, effects that can be demonstrated in minutes to hours. Second, insulin resistance can develop in cells or animals without discernible or reproducible changes in insulin signaling and, conversely, animals with an engineered insulin signaling deficiency do not uniformly develop insulin resistance under normal conditions [9–13].

**Could Nuclear Mechanisms Play a Role in Insulin Resistance?**

There are compelling reasons to suspect that nuclear events, defined here as processes such as transcriptional and epigenetic regulation taking place in the nucleus, play a role in the development of insulin resistance. First, insulin sensitivity can be enhanced with drugs that primarily act through transcription factor targets, such as thiazolidinediones (TZDs), an activator of PPARγ [14,15] and glucocorticoids, which activate the glucocorticoid receptor. Second, drugs that affect chromatin remodeling, such as certain histone deacetylase (HDAC) inhibitors, are known to affect insulin sensitivity in cells, animal models, and humans [16]. Third, mice with genetic alterations in chromatin modifying enzymes, such as Jhdm2a and Ehmt1, develop obesity and insulin resistance [17–19]. Finally, and perhaps most compelling, there is a very large body of literature that indicates that the risk of developing insulin resistance in later life is strongly affected by nutritional conditions experienced in utero. For example, pregnant rodents that undergo caloric restriction give birth to offspring that have a significantly greater chance of developing insulin resistance as adults [20]. The same phenomenon has been reported in human populations, as with offspring of Dutch women who were pregnant during the ‘hunger winter’ of 1944–1945 [21]. Such examples of ‘metabolic memory’ are predicted to have an epigenetic basis, and there are data that support this mechanism directly, such as altered histone modification at the Slc2a4 (Glut4) locus in the offspring of calorically restricted rats [22].

Systemic insulin sensitivity is determined by the interactions of several different tissues and cell types [23,24]. Classically, most people think of insulin action at the liver, muscle, and adipose tissue, as the organs most responsible for insulin-dependent glucose production and disposal. Recent data, however, suggest a significant role for other tissues as well, notably the brain, which does not take up glucose in response to insulin but which can regulate the actions of insulin in classical target tissues indirectly [25]. Cells of the immune system are another example; although not insulin sensitive themselves, their number and activation state has a major effect on insulin action locally (in adipose tissue, for example) and systemically [23,26]. Thus, a transcription factor can cause insulin resistance indirectly via effects on macrophage polarization, for example [27].

Finally, we should keep in mind that several (non-mutually exclusive) nuclear events may be involved, including changes in transcription factor expression, binding, post-translational modifications, and protein–protein interactions with cofactors/chromatin modifiers. In all of these scenarios, the relevant output is altered target gene expression. These target genes could be obvious, such as those encoding classic insulin signaling proteins, but may also be obscure. For example, several genes were recently identified in adipocytes that are dysregulated in insulin-resistant states and which cause insulin resistance when overexpressed; none of these genes participates in a pathway known to be involved in insulin action [9]. In the next section, we
highlight three separate transcription factors and cofactors with significant actions on insulin sensitivity, and we discuss possible mechanisms by which they exert these effects. Although we focus on a few notable examples, many others exist, including transcription factors, nuclear cofactors, and chromatin modifying enzymes; some of these are indicated in Table 1.

**Nuclear Receptors as a Paradigm for Affecting Insulin Action in Adipose Tissue**

Among transcription factors, the nuclear receptor (NR) superfamily plays a special role in regulating insulin sensitivity, given that they are activated by small ligands that are often fatty acid derivatives or other nutritional byproducts. NRs thus provide a direct link between environmental conditions and the genome. Accordingly, several NRs have been implicated in the regulation of insulin action, most notably PPARγ and the glucocorticoid receptor (GR). Other examples of NRs that have been implicated in insulin resistance include VDR, LXR, FXR, and LRH-1.

**Peroxisome Proliferator-Activated Receptor γ (PPARγ)**
The NR best associated with insulin action is PPARγ, most notably via its position as the target of TZDs, which are used clinically as insulin sensitizers. Although originally identified as a dominant regulator of adipogenesis, PPARγ is now known to be expressed in many different tissues and cell types (although to a lower degree than adipose tissue) and to be involved in processes as distinct as lipid accumulation, glucose homeostasis, skeletal homeostasis, and inflammation [14,15]. This broad tissue distribution has provoked numerous studies attempting to determine which site of action is most relevant for the insulin-sensitizing properties of PPARγ (Figure 1).

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**Table 1. Selected Additional Transcription Factors, Cofactors, and Epigenomic Modifiers that Have an Impact on Insulin Sensitivity**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Tissue</th>
<th>Effect on Insulin Action</th>
<th>Mechanism</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcription Factor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LXRα</td>
<td>White adipose tissue (WAT)</td>
<td>Increased</td>
<td>Enhances Glut4 expression</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Increased</td>
<td>Suppresses gluconeogenesis</td>
<td>[85]</td>
</tr>
<tr>
<td>VDR</td>
<td>Macrophage</td>
<td>Increased</td>
<td>Promotes M2 macrophage polarization</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>Adipocyte</td>
<td>Decreased</td>
<td>Inhibits glucose uptake</td>
<td>[9]</td>
</tr>
<tr>
<td>ChREBP</td>
<td>WAT</td>
<td>Increased</td>
<td>Promotes glucose transport</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Increased</td>
<td>Promotes lipogenesis and glycolysis</td>
<td>[88]</td>
</tr>
<tr>
<td>LRH-1</td>
<td>Liver</td>
<td>Increased</td>
<td>Promotes glucose uptake</td>
<td>[89]</td>
</tr>
<tr>
<td><strong>Transcription Cofactor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRC1, p/CIP</td>
<td>WAT, muscle</td>
<td>Decreased</td>
<td>Suppresses insulin signaling</td>
<td>[90]</td>
</tr>
<tr>
<td>NCoR</td>
<td>WAT</td>
<td>Decreased</td>
<td>Suppresses PPARγ action</td>
<td>[91]</td>
</tr>
<tr>
<td>CRTC2</td>
<td>Liver</td>
<td>Decreased</td>
<td>Promotes gluconeogenesis</td>
<td>[92]</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Liver</td>
<td>Decreased</td>
<td>Promotes gluconeogenesis</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>Increased</td>
<td>Enhances Glut4 expression</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>WAT/BAT</td>
<td>Increased</td>
<td>Enhances browning/thermogenesis</td>
<td>[95]</td>
</tr>
<tr>
<td><strong>Epigenomic Modifier</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jhdm2a</td>
<td>WAT</td>
<td>Increased</td>
<td>Promotes browning</td>
<td>[19]</td>
</tr>
<tr>
<td>Sirt1</td>
<td>WAT</td>
<td>Increased</td>
<td>Inhibits inflammation</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>Increased</td>
<td>Enhances glucose uptake</td>
<td>[97]</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Increased</td>
<td>Inhibits endoplasmic reticulum stress</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>Macrophage</td>
<td>Increased</td>
<td>Inhibits inflammation</td>
<td>[99]</td>
</tr>
</tbody>
</table>
Somewhat amazingly, specific deletion of PPARγ in many different cell types, including adipose tissue, muscle, macrophages, and brain alters glucose homeostasis, and reduces the full activity of TZDs. The preponderance of evidence, however, suggests that adipose tissue is the major site of action for the insulin-sensitizing actions of PPARγ [28–30]. Mice lacking PPARγ in liver respond to TZDs normally unless adipose tissue is also defective [28], and liver- and muscle-specific PPARγ knockout mice were shown to display insulin resistance, although to a much lesser degree than adipose-specific knockout animals, and show variable responses to TZDs [31,32]. A role for PPARγ has been speculated to be important in pancreatic β cells, but while TZDs enhance insulin secretion from isolated islets in a PPARγ-dependent manner, mice lacking islet PPARγ have intact glucose homeostasis and respond normally to TZDs [33].

Immune cells are another likely site of PPARγ action. PPARγ is highly expressed in macrophages, where it promotes alternative M2 polarization of macrophages [34,35]. Macrophage-specific deletion of PPARγ conferred protection from diet-induced insulin resistance, although this is still improved by rosiglitazone [36,37]. A more recent study has provided evidence that PPARγ in a subset of regulatory T (Treg) cells plays a critical insulin-sensitizing role, as animals with PPARγ deletion in this cell type show no response to pioglitazone [38] in glucose metabolism and insulin sensitivity. Lastly, there are indications that the weight gain associated with PPARγ activation is mediated partially through actions in the brain [39,40]. However, rosiglitazone still improved whole-body (but not hepatic) insulin sensitivity in mice lacking PPARγ in the brain [39], indicating that central nervous system (CNS) effects may account for some but not all the metabolic effects of TZDs.

**What is the Mechanism through which PPARγ Improves Insulin Sensitivity?**

Several mechanisms have been proposed to explain the basis of the PPARγ-dependent insulin-sensitizing effect. In adipose tissue, the lipogenic and antilipolytic actions of PPARγ exert a so-called ‘lipid steal’ effect, in which fatty acids and other lipids are sequestered safely in adipose

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**Figure 1. Multiorgan Effects of PPARγ, GR, and FoxO1 and Their Impact on Insulin Sensitivity.** Multiple sites of action for these transcription factors contribute to their effects on metabolism and glycemic control. See text for details. Abbreviations: PPARγ, peroxisome proliferator-activated receptor γ; GR, glucocorticoid receptor.

<table>
<thead>
<tr>
<th>PPARγ</th>
<th>GR</th>
<th>FoxO1</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Browning</td>
<td>↓ Lipogenesis</td>
<td>↓ Adipokine secretion</td>
</tr>
<tr>
<td>↓ Glucose uptake</td>
<td>↓ Glucose uptake</td>
<td>↓ Glucose uptake</td>
</tr>
<tr>
<td>↓ Insulin signaling</td>
<td>↓ Insulin signaling</td>
<td>↓ Insulin signaling</td>
</tr>
<tr>
<td>↓ Lipid storage</td>
<td>↓ Lipid storage</td>
<td>↓ Adipocyte differentiation</td>
</tr>
<tr>
<td>↓ Adipokine secretion</td>
<td>↓ Adipocyte differentiation</td>
<td>↓ Adipocyte differentiation</td>
</tr>
<tr>
<td>↓ Food intake</td>
<td>↓ Food intake</td>
<td>↓ Body weight</td>
</tr>
<tr>
<td>↓ Inflammation</td>
<td>↓ Inflammation</td>
<td>↓ Apoptosis</td>
</tr>
</tbody>
</table>

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[31,32]...
tissue. This counters the damaging actions of potentially toxic lipids in other tissues, which has been postulated to account for insulin resistance [41]. TZDs also exert a direct glucose-lowering effect associated with improved insulin signal transduction in muscle and adipose [42,43]. Yet another mechanism, at least in rodents, involves the ability of TZDs to promote ‘browning’ of white fat [44–46], which leads to increased energy expenditure and improved whole-body metabolism. PPARγ also promotes both the expression and secretion of adiponectin, a potent insulin-sensitizing hormone [47]. Finally, the adipogenic actions of PPARγ may also mediate some of the insulin-sensitizing effects; the reason for this may involve a variation of the ‘lipid steal’ hypothesis (with new adipocytes contributing to the safe storage of toxic lipid species) and may also reflect a healthier adipokine and anti-inflammatory profile in newly formed, smaller adipocytes than in large, hypoxic adipocytes.

PPARγ is a transcription factor, of course, so its proximal actions are believed to involve alterations in gene expression, both positive and negative (Figure 2). Positive targets include adiponectin, as well as a variety of lipogenic enzymes, transporters, and signal transduction intermediates. Negatively regulated genes in adipose tissue with relevance to insulin sensitivity include RBP4, resistin, and a variety of cytokines [14]. To date, several studies have been performed to comprehensively identify PPARγ-dependent gene expression and binding events

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Binding arrangement</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, Muscle, Liver</td>
<td>TZD</td>
<td>Lipid and glucose metabolism (e.g., Adipoq, Slc2a4, CD36)</td>
</tr>
<tr>
<td></td>
<td>PPARγ, RXR</td>
<td></td>
</tr>
<tr>
<td>Immune cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possibly other cells</td>
<td>TZD</td>
<td>Inflammation (e.g., Tnfa, Iil6, Ifng)</td>
</tr>
<tr>
<td></td>
<td>PPARγ, TF</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>GC, GC</td>
<td>Gluconeogenesis (e.g., G6pc, Pck1)</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>Lipogenesis (e.g., Scd2, Lpin1)</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>Negative regulator of insulin signaling (p85a)</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>Lipolysis (e.g., Angptl4)</td>
</tr>
<tr>
<td></td>
<td>NFκB, AP1, etc.</td>
<td>Inflammation (e.g., Dusp1, IκBa)</td>
</tr>
<tr>
<td>All</td>
<td>GR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NFκB, AP1, etc.</td>
<td>Inflammation (e.g., Tnfa, Iil6)</td>
</tr>
</tbody>
</table>

Figure 2. Different Modes of Transcriptional Regulation by PPARγ and GR in Insulin Sensitivity. In fat, muscle, and liver, ligand activation of PPARγ induces heterodimerization with RXR, allowing DNA binding to specific response elements and subsequent transcriptional activation. Negative regulation by PPARγ, as occurs with proinflammatory genes in immune cells, is incompletely understood but likely involves ‘tethering’ via direct interactions with other transcription factors. Ligand binding of GR induces homodimerization of GR, allowing DNA binding to activate and repress GR target genes in multiple tissues. Similar to PPARγ, GR can exert transrepression via tethering to other transcription factors to repress genes in inflammation. Abbreviations: PPARγ, peroxisome proliferator-activated receptor γ; GR, glucocorticoid receptor; RXR, retinoid X receptor.
with and without TZD treatment (reviewed in [48]). However, our knowledge of the core transcriptional and epigenetic events through which PPARγ regulates insulin sensitivity remains incomplete.

**Is Targeting PPARγ Still a Viable Therapeutic Strategy?**

Despite the well-known antidiabetic effects of TZDs, their clinical use has been restricted as a result of serious adverse effects such as weight gain, plasma volume expansion, bladder cancer, and increased risk of cardiac heart failure, although some of these fears may have been overblown [15]. Nonetheless, the negative reports of TZD adverse effects led to a pronounced chilling of the PPARγ targeting programs of most major pharmaceutical companies. It remains to be seen whether the more reassuring data that have emerged recently will reinvigorate these activities. One promising area for future drug discovery relates to altering post-translational modifications of PPARγ to help treat metabolic disease. For example, preventing PPARγ phosphorylation at S112 preserved insulin sensitivity in response to a high-fat diet, associated with reduced adipose hypertrophy, increased adiponectin, and reduced free fatty acid levels [49]. Recent studies have also demonstrated that Erk/CDK5-dependent PPARγ phosphorylation at S273 regulates a selective set of PPARγ targets that are highly relevant to metabolic regulation [50,51]. Furthermore, PPARγ agonists and MEK inhibitors that block this phosphorylation event exert antidiabetic effects without causing weight gain or hypoglycemia [52]. Other potentially interesting post-translational modifications of PPARγ that may be amenable to therapeutic targeting include SUMOylation at Lys365 and Lys107, which mediates transrepression of inflammatory response genes, especially in macrophages [37]. SUMOylation at Lys107 can also lead to degradation of PPARγ, and this can be blocked by FGF21; this may mediate the insulin-sensitizing actions of that hormone [53]. Sirt1-mediated deacetylation of PPARγ [45] also leads to beneficial metabolic effects in metabolic syndrome.

**Glucocorticoid Receptor (GR)**

Another NR transcription factor with pronounced effects on insulin action is the glucocorticoid receptor (GR, encoded by NR3C1), which mediates the metabolic effects of endogenous and synthetic glucocorticoids [54,55]. Activation of the hypothalamic–pituitary–adrenal (HPA) axis by stress, including fasting, induces glucocorticoid synthesis and secretion from the adrenal cortex [55]. Glucocorticoids exert a wide range of actions on metabolic tissues, the net effect of which is to raise blood sugar as required by the brain. Prolonged GR activation is associated with metabolic dysregulation, including insulin resistance, as occurs in Cushing’s syndrome [56] or after long-term exposure to pharmacological doses of glucocorticoids [57].

**How Does GR Promote Insulin Resistance at the Molecular Level?**

Glucocorticoids bind to the ligand-binding domain of the GR in the cytoplasm, where it is sequestered by binding to Hsp90, Hsp70, and other chaperone proteins, and cause it to translocate to the nucleus (Figure 2). Once in the nucleus, GR can alter gene expression in one of two ways. First, it can bind directly to glucocorticoid response elements (GREs) in the enhancers and promoters of various genes as a homodimer. Alternatively, GR can bind to other transcription factors as part of a ‘tethered’ complex that does not contact DNA directly. Both mechanisms can be used to promote gene activation or repression [55]. There has been a long-standing belief that the beneficial actions of glucocorticoids, such as suppression of inflammation, primarily utilize the tethering model, and that adverse consequences of steroid action, including insulin resistance, require homodimerization and direct DNA binding. Recent studies call this into question, as mice that contain a mutation in the homodimerization domain of GR that does not affect tethering display reduced insulin sensitivity at baseline, and still develop full insulin resistance upon treatment with dexamethasone [58]. It is true, however, that GR does directly bind and regulate a number of genes involved in gluconeogenesis, lipogenesis, and insulin signal transduction [55,59]. In addition to these genomic actions of GR, nongenomic effects have also
been proposed to contribute to glucocorticoid-induced insulin resistance; dexamethasone can inhibit insulin signaling very rapidly in cultured adipocytes in a manner that is not reversed by inhibiting transcription, for example [60].

What Tissues Are Critical for GR to Regulate Insulin Sensitivity?
Most cells express the GR, including adipose tissue, muscle, and liver. In liver, GR activation promotes hepatic glucose output, an effect considered to be attributable to direct regulation of key gluconeogenic genes such as Pck1 and G6pc. Thus, mice with liver-specific GR knockout or knock-down have reduced hepatic glucose output [61] (Figure 1). It is worth noting, however, that GR may also control gluconeogenesis via extrahepatic sites such as the hypothalamus, as direct injection of dexamethasone into the arcuate nucleus causes hepatic insulin resistance [62]. These actions are independent of, but enhanced by, the increased food intake and weight gain associated with chronic glucocorticoid administration [63].

In skeletal muscle, chronic GR activation contributes to insulin resistance by inhibiting protein synthesis and promoting proteolysis, thus releasing amino acids that are used as substrate for glucose production. Glucocorticoids also inhibit insulin-stimulated glucose uptake. The underlying mechanism for this is not well understood, but a recent ChIP-Seq study has shown that GR directly targets genes involved in insulin signaling [64]. GR mRNA levels in the skeletal muscle of diabetic patients correlates with the degree of insulin resistance, and expression normalizes following the administration of insulin sensitizers [65].

Although the precise role of adipose tissue GR in glucose metabolism and insulin resistance remains to be addressed in vivo, many studies have shown that activation of GR suppresses insulin-stimulated glucose uptake assay in cultured adipocytes [6,9]. GR causes this, in part, by directly regulating the expression of several downstream effector genes (e.g., Vdr, Tmem176a, Serpina3n, Lcn2), which reduce insulin-stimulated glucose uptake in as yet unclear ways. Interestingly, adipocyte GR can be activated by TNF-α in a partially ligand-independent way, representing an extraordinary example of an anti-inflammatory transcription factor mediating the metabolic effect of a proinflammatory cytokine [9].

Control of Insulin Sensitivity by PGC-1α and FOXO1
FOXO transcription factors are key regulators of metabolism and are canonical mediators of insulin-dependent changes in gene expression (reviewed in [66]). There are four mammalian FoxO genes (FoxO1, FoxO3a, FoxO4, and FoxO6), which have overlapping functions (Arden, 2009). Although no common polymorphisms in the FOXO1 locus have been directly linked to insulin resistance or diabetes by large-scale genome-wide association studies (GWAS), a functional network analysis of the genes nearest GWAS signals for glycemic traits implicate FOXO1 as a shared interactor with multiple GWAS candidate proteins [67].

In the liver, insulin promotes the accumulation of lipid and represses gluconeogenesis; the latter is the major determinant of systemic glucose homeostasis in the fasted state. Among the many factors that regulate gluconeogenesis, the transcription factor FOXO1 and the cofactor PGC-1α have been identified as having a major role (Figure 1). Liver-specific triple knockout of FOXO1, FOXO3a, and FOXO4 show increased fasting hypoglycemia, increased glucose tolerance, and enhanced insulin sensitivity with decreased plasma insulin levels; comparisons with single knockouts suggest all three work synergistically to regulate hepatic insulin sensitivity [68]. Whole-body FOXO6 knockouts also show decreased hepatic glucose production and enhanced insulin sensitivity [69].

FOXO1 is thought to primarily affect insulin sensitivity in adipose tissue via inhibition of adipocyte differentiation, although transgenic expression of a dominant-negative FOXO1 in mature
adipocytes improves glucose and insulin tolerance and increases energy expenditure in mice on a high-fat diet [70]. In the pancreas, FOXO1 is involved in β cell dysfunction via multiple mechanisms including suppression of β cell proliferation, mediating oxidative, endoplasmic reticulum, and hypoxic stress, and increasing apoptosis (reviewed in [66]). FOXO1 has also been shown to mediate insulin-regulated activity of hypothalamic neurons, with constitutively active FOXO1 causing hyperphagia, increased body weight, and inhibition of leptin action. Mice with Agrp neuron-specific deletion of FOXO1 are lean with reduced food intake and suppressed hepatic glucose production [71].

FOXO factors generally, and FOXO1 in particular, are regulated by a variety of post-translational modifications, including phosphorylation and acetylation (reviewed in [72]). In its nonphosphorylated state, FOXO1 localizes to the nucleus, where it binds its cognate motif and drives expression of key insulin-regulated genes, such as G6pc (encoding glucose 6-phosphatase) (Figure 3). Insulin causes Akt-mediated phosphorylation of FOXO1 at residues T24, S256, and S319, resulting in its exclusion from the nucleus, where it is subsequently ubiquitinated and degraded. FOXO proteins are also a key element of the oxidative stress response pathway, which causes insulin resistance in multiple tissues. ROS induce JNK-mediated phosphorylation of FOXO1, driving increased transcriptional activation of target genes, which include antioxidant scavengers [73]. Transcriptional profiling of cells expressing a nonphosphorylatable mutant FOXO1 display upregulated ROS response genes [74], while the vast majority of other FOXO1 targets are unchanged. FOXO1 is also activated by AMP-activated Protein Kinase (AMPK) phosphorylation, and its activation inhibits gluconeogenesis in the liver and glucose oxidation in the muscle. In response to oxidative stress, β-catenins also directly interact with FOXO1 to increase its transcriptional activity [75,76]. Such β-catenin/FOXO1 interactions in the liver have been shown to contribute to regulation of gluconeogenic gene expression and glucose homeostasis [77].

FOXO1 activity is also modulated by acetylation, serving as a direct target of the histone acetyltransferases CBP and p300, and the HDACs SIRT1 and SIRT2. The effect of acetylation on FOXO1 activity appears to be context-dependent, with data showing positive, negative, and dual effects on transactivation (reviewed in [78]). FOXO1 acetylation status is determined by the relative balance of protein acetylases (CBP, p300, and PCAF) and deacetylases (Sir2/Sirt family).

Figure 3. Transcriptional Activity of FOXO1 and PGC-1α in Hepatic Glucose Production. FOXO1 is a key driver of gluconeogenic gene expression (e.g., Pdk1 and G6pc) in the liver. Its activity is regulated by a variety of factors, including phosphorylation by Akt, which reduces FOXO1 levels in the nucleus, and by phosphorylation via AMPK, which promotes its activity. Other post-translational modifications, such as O-linked GlcNAcylation and acetylation, also affect the activity of FOXO1, as does interaction with the cofactor PGC-1α. See text for more details. Abbreviation: AMPK, AMP-activated Protein Kinase.
FOXO1 acetylation promotes phosphorylation by Akt and inhibits DNA binding [79]. Meanwhile, SIRT1-mediated deacetylation of FOXO family proteins, as occurs in response to ROS, promotes nuclear translocation and gluconeogenic gene expression, even in the face of Akt activation [80]. FOXO factors can also be activated by O-GlcNAcylation following increased oxidative stress; this modification is increased in diabetic livers and is associated with increased FOXO-driven expression of gluconeogenic and ROS detoxifying genes [81].

In the liver, the coactivator PGC-1α is a key insulin-responsive transcriptional regulator of gluconeogenesis. PGC-1α is induced in the liver on fasting, is elevated in models of insulin resistance, and can activate the entire transcriptional profile of gluconeogenesis [82,83]. The induction of gluconeogenesis by PGC-1α is mediated via direct interaction with FOXO1; Akt-mediated phosphorylation and expulsion of FOXO1 from the nucleus disrupts the FOXO1–PGC-1α interaction, thereby suppressing gluconeogenesis [84].

Concluding Remarks

Insulin sensitivity is a carefully regulated process that goes awry in many different pathophysiological states. Its role as a major driver of type 2 diabetes means that we must understand its antecedents and its consequences to develop better and more rational targets for therapeutic interventions (see Outstanding Questions). Numerous theories have emerged to explain how insulin resistance develops and progresses, most of which involve changes in insulin signal transduction, or other processes that center on the cytosol, or organelles such as mitochondria and the endoplasmic reticulum. Here we have endeavored to draw attention to transcriptional and epigenetic events that play an equally important role. These pathways involve a wide variety of organs and cell types and numerous molecular players, including transcription factors, cofactors, and chromatin-modifying enzymes. They utilize a widely varying repertoire of mechanisms that include direct effects on the expression of insulin signaling components, repression or enhancement of inflammation, effects on cellular differentiation, and actions on targets with as yet unspecified roles in insulin sensitivity. Some of these pathways, such as those involving nuclear hormone receptors or chromatin-modifying enzymes, are inherently ‘druggable’, while other factors may prove to be more resistant to pharmacological intervention. Regardless, a full exploitation of nuclear mechanisms and their downstream effectors will broaden our understanding of insulin resistance and should enable the identification of novel drug targets.

References


Outstanding Questions

What are the key gene targets that mediate the effects of relevant transcription factors and cofactors on insulin action? Does each transcription factor work on its own set of targets, or are there a common set of critical genes that are coregulated by multiple factors?

How are transcriptional effects in different tissues, such as liver, adipose, muscle, brain, and immune cells, coordinated so that they produce a unified effect on metabolism?

What are the molecular mechanisms through which transcription factors regulate genes involved in insulin resistance? Are core subsets of cofactors and chromatin marks utilized?

Other than PPARγ agonism, can transcriptional and epigenomic pathways be manipulated to improve insulin sensitivity in patients? How many of these pathways are ‘druggable’, and how can we achieve tissue-selective effects?
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