Key Symposium

Journal of INTERNAL MEDICINE

doi: 10.1111/joim.12547

Epigenomic and transcriptional control of insulin resistance

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Abstract. Rosen ED (Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA). Epigenomic and transcriptional control of insulin resistance. (Key Symposium). *J Intern Med* 2016; **280**: 443–456.

Insulin resistance is one of the defining features of type 2 diabetes and the metabolic syndrome and accompanies many other clinical conditions, ranging from obesity to lipodystrophy to glucocorticoid excess. Extraordinary efforts have gone into defining the mechanisms that underlie insulin resistance, with most attention focused on altered signalling as well as mitochondrial and endoplasmic reticulum stress. Here, nuclear mechanisms of insulin resistance, including transcriptional and epigenomic effects, will be discussed. Three levels of control involving transcription factors, transcriptional cofactors, and chromatin-modifying enzymes will be considered. Well-studied examples of the first include PPAR-γ in adipose tissue and the glucocorticoid receptor and FoxO1 in a variety of insulin-sensitive tissues. These proteins work in concert with cofactors such as PGC-1α and CRTC2, and chromatin-modifying enzymes including DNA methyltransferases and histone acetyltransferases, to regulate key genes that promote insulin-stimulated glucose uptake, gluconeogenesis or other pathways that affect systemic insulin action. Furthermore, genetic variation associated with increased risk of type 2 diabetes is often related to altered transcription factor binding, either by affecting the transcription factor itself, or more commonly by changing the binding affinity of a noncoding regulatory region. Finally, several avenues for therapeutic exploitation in the battle against metabolic disease will be discussed, including small-molecule inhibitors and activators of these factors and their related pathways.

Keywords: adipocyte, chromatin-modifying enzyme, insulin resistance, transcription factor, transcriptional cofactor, type 2 diabetes.

Introduction

The world has reached an inflection point where, for the first time in human history, more suffering and death are caused by diseases of overnutrition than by diseases of undernutrition [1]. This is manifested by the extraordinary increase in the prevalence of obesity and its complications, most notably type 2 diabetes (T2D). Astonishingly, more than half of all adult Americans have prediabetes, with almost 15% afflicted by full-blown T2D [2]. Insulin resistance is a *sine qua non* of T2D, and defines the state in which cells, tissues, or whole organisms no longer respond appropriately to either endogenous or exogenous insulin. Obesity-associated insulin resistance occurs when metabolic cells in the periphery, particularly adipocytes, hepatocytes and myocytes, become dysfunctional in the face of nutritional stress. It should be noted, however, that other types of cellular stress are also associated with insulin resistance. For example, trauma, burns, starvation, inflammation, glucocorticoid excess, some malignancies and even pregnancy are all accompanied by diminished response to insulin [3]. Presumably, this response to stress has adaptive value, perhaps by sparing calories from being taken up by adipocytes and stored so that they can be utilized preferentially by tissues in need.

Most analyses of insulin resistance have focused on defects in the insulin signalling pathway, a complex and incompletely understood network of interconnected kinases and adaptor proteins that transmits information from the insulin receptor on the surface of the cell to various effectors within the cell, resulting ultimately in a suite of anabolic actions including adipocyte differentiation, glucose uptake and lipid and protein synthesis. It has long been assumed that clinically relevant insulin resistance is the result of altered insulin signalling, and there are data to support this idea. Many genetically engineered mice that lack key signalling

intermediates are insulin resistant, and reduced levels of some key signalling proteins have been noted in the tissues of insulin-resistant patients [3–5]. Other pathways that have been proposed to cause or contribute to insulin resistance, such as inflammation, endoplasmic reticulum stress and oxidative stress, are ultimately hypothesized to converge on insulin signalling as their final effector mechanism [6–9]. Recently, however, this ultimate dependency on signalling abnormalities has been called into question. There are now several cellular and animal models in which insulin resistance can develop in the absence of any discernible changes in signalling activity [10–14].

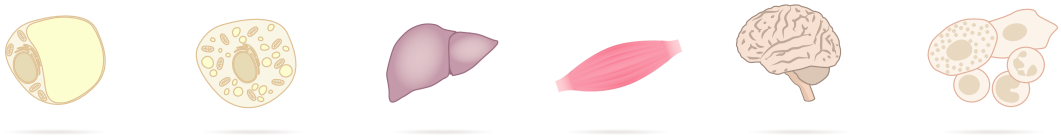
These observations suggest that additional pathways must also be involved. In this review, this possibility is discussed more fully with specific reference to mechanisms operating within the cellular nucleus. Such pathways, as defined here, ultimately affect gene expression in a physiologically relevant cell type, but as will be shown, the defect in insulin resistance can occur at the level of the transcription factor, transcriptional cofactors (i.e. proteins that regulate gene expression via indirect actions on DNA) or chromatin-modifying enzymes, which alter the receptivity of DNA for transcription. Given the plethora of well-supported mechanistic theories that underlie insulin resistance, why is it necessary to invoke such nuclear activities? There are several different reasons. First, and perhaps most importantly, there is a large body of data that links *in utero* nutritional exposures to the development of obesity and insulin resistance later in life. This has been observed in a large number of rodent models [15], and also in certain experiments of human history. One of these, the so-called Dutch Hunger Winter of 1944–1945, was characterized by a significant increase in metabolic disease in the adult offspring of women who were exposed to near-starvation conditions whilst pregnant [16]. Such examples of multigenerational and transgenerational passage of disease risk are considered to be paragons of epigenetic transmission and have been associated with epigenomic factors including altered DNA methylation, histone modifications and noncoding RNA. Another link between transcriptional and epigenetic factors and insulin resistance is found in the clinical utility of thiazolidinedione (TZD) drugs to treat insulin resistance; these agents are direct agonists of the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) [17]. Other small molecules targeting chromatin-modifying enzymes also affect systemic insulin sensitivity, including the histone deacetylase (HDAC) inhibitor valproic acid [18]. Systemic insulin sensitivity is also affected in mice with targeted ablation of several chromatin-modifying enzymes [19–21].

Systemic insulin sensitivity is controlled by multiple cells, tissues and molecular pathways

Systemic insulin resistance can result from an insult to any of several different tissues and organs (Fig. 1) [8, 22, 23]. Skeletal muscle has been an area of particular focus as the primary site for glucose disposal after a meal. Surprisingly, however, complete loss of insulin receptors in mouse skeletal muscle does not cause overt hyperglycaemia [24]. Other peripheral organs with a major role in systemic insulin action include the liver and adipose tissue, both of which have a dominant role in systemic glucose homeostasis. Recent developments suggest that other cell types may also be of critical importance. For example, macrophages, eosinophils, T and B lymphocytes and other immune cells have all been implicated in insulin sensitivity through their effects on adipose tissue [25, 26]. Brown adipose tissue takes up huge quantities of glucose and fatty acids as fuel for heat generation; the overall activity of brown adipose tissue is a major determinant of insulin sensitivity in rodents and may also have implications for humans [27]. Finally, the central nervous system has been implicated in the control of insulin responsiveness in the liver and other organs, and should be considered an additional site where transcriptional control may affect systemic glucose homeostasis [28].

Transcriptional and epigenetic events must ultimately affect insulin sensitivity via changes in gene expression. Thus, a key question is: Which genes are most relevant to the development of insulin resistance? The most obvious candidates would be genes that encode proteins known to be involved in insulin signalling directly, such as the insulin receptor, IRS-1, Akt2 and others. Many of these genes are, in fact, under modulatable transcriptional control. *Slc2a4*, which encodes the insulin-stimulated glucose transporter Glut4, provides such an example, as its mRNA levels are dramatically reduced in obese human adipose tissue (but not muscle) [29]. Other genes in the insulin signalling pathway are regulated by transcription factors such as C/EBPα, but their levels tend to be less sensitive to changes in nutritional status or disease state [30].

Multiorgan control of insulin sensitivity



	WAT	BAT	Liver	Muscle	CNS	Immune cells
Process	Glucose uptake Adipokine secretion	Glucose uptake Adipokine secretion Fatty acid oxidation	Glucose production Hepatokine secretion	Glucose uptake Myokine secretion	Regulation of appetite Regulation of energy expenditure	Inflammation
Genes	Glut 4 IR IRS LEP ADIPOQ	Glut 4 IR IRS1	IR PCK1 G6pc FGF21	Glut4 IR IRS1 FGF21 FNDC5	AGRP POMC LEPR MC4R	IL-6 TNF-α IL-1β
TFs	PPAR-γ CEBP-α EBF1 IRF4 GR	PPAR-γ CEBP-β IRF4 PPAR-α	CREB FOXO1 GR	MEF2 KLF15 GR	FOXO1 STAT3	IRF3 IRF4 NF-κB GR

Fig. 1 *Insulin action and glycaemic status is controlled by many different tissues working together, including white adipose tissue (WAT) and brown adipose tissue (BAT), liver, muscle, immune cells and the central nervous system (CNS). Each of these tissues regulates different processes with an impact on glucose homeostasis, mediated by a complex series of genes and transcription factors (TFs), some of which are shown.*

Other gene expression events linked to insulin sensitivity may be more indirect. Obviously, almost any gene expression event in any tissue that results in altered body weight will have a corresponding effect on insulin action. This includes transcriptional activity in the hypothalamus or brown adipose tissue. Furthermore, the close relationship between inflammation and insulin resistance suggests that altering transcription in immune cells may impact systemic glucose homeostasis. As one example, the transcription factor IRF4 promotes alternative (M2) activation of macrophages; loss of IRF4 in macrophages alters the M1/M2 ratio and worsens insulin resistance in the setting of obesity [31].

Basic principles of epigenomic control of transcription

Regulation of gene expression is a complex, multifactorial process, but for the purposes of this review, genes can be considered to have various regulatory elements, most notably a promoter that sits immediately upstream of the transcription start site (TSS), and several enhancers that reside farther away (i.e. upstream of the TSS, downstream of the gene or within an intron) (Fig. 2). Most genes have many enhancers, but most enhancers regulate one or a few genes [32]. Enhancers serve as docking sites for transcription factors, proteins that bind to DNA in a sequence-specific fashion. Transcription factors act in large complexes that include proteins loosely designated as 'cofactors'; these proteins do not bind DNA by themselves, but instead regulate gene expression indirectly by modifying the activity of a bound transcription factor. In general, cofactors are either positive (i.e. co-activators) or negative (i.e. corepressors) effectors of gene expression. Thus, whether a transcriptional complex induces or represses gene expression is a reflection of the net balance of co-activators and corepressors bound at any given time; this is a dynamic process regulated by signalling events and nutritional status. Finally, the chromatin at the site of the gene being expressed (or repressed) has to be altered to enable more efficient transcription; this involves a series of chromatin-modifying enzymes that alter the structure of the nucleosome by changing the

post-translational modifications on local histones [33, 34]. These enzymes include the so-called epigenomic 'writers', which deposit post-translational marks on histones and other chromatin-associated proteins. One example is histone acetyltransferases (HATs), which tend to open up chromatin and enhance transcription at that location. Other examples include histone methyltransferases, which can have a variable effect on gene expression depending upon which amino acid residue of which histone is modified; thus, adding methyl groups to histone 3 at the lysine 4 (H3K4) position increases transcription, whereas adding methyl groups to histone 3 lysine 27 (H3K27) has the opposite effect. There are also epigenomic 'erasers', which remove these marks, including HDACs and demethylases. Other proteins affect the state of the chromatin at enhancers and promoters, notably including DNA methyltransferases, which promote the covalent modification of cytosine to 5-methylcytosine, which also affects gene expression. This very simple primer belies an extraordinarily complex area of biology with new information constantly emerging; the interested reader is referred to several excellent recent reviews for more information [35–37].

Regulation at the level of the transcription factor

PPAR-γ

Many transcription factors affect insulin sensitivity at the cellular, tissue and organismal level. As described above, such factors can affect many different gene expression programs in a variety of cell types. Several nuclear hormone receptors (NHRs), for example, have been implicated in the control of insulin sensitivity. The NHRs represent a superfamily of transcription factors that includes steroid, retinoid and thyroid hormone receptors, amongst others; the intrinsic ability of these proteins to respond to small-molecule ligands makes them uniquely suited amongst transcription factors to transduce environmental signals in the form of hormones or nutritional intermediates such as fatty acids.

One well-known example is PPAR-γ which is the physiological target of an as yet unidentified endogenous lipid species, in addition to being the pharmacological target of TZD drugs such as rosiglitazone and pioglitazone. PPAR-γ is expressed in many different tissues and has been implicated in a variety of biological processes including adipogenesis, bone remodelling and inflammation [17, 38]. The major site of PPAR-γ expression and action, however, is believed to be the adipocyte, where it serves as a 'master regulator' of differentiation, lipogenesis and insulin sensitivity. PPAR-γ binds to a large number of genomic sites in adipocytes, and in the presence of ligand, it activates or represses local gene expression [39]. The precise mechanisms by which PPAR-γ activation improves insulin resistance in fat are not entirely clear, but almost certainly include the promotion of adiponectin synthesis and release, repression of adipokines that inhibit insulin action (e.g. RBP4 and resistin), and the induction of a large number of genes encoding glucose and fatty acid transporters, lipid synthesis enzymes and lipid droplet proteins, the net effect of which is to partition fatty acids and glucose away from other tissues and to store these nutrients as neutral lipids in a 'safe' environment [40]. TZDs and PPAR-γ also cause enhanced 'browning' of white adipose tissue, at

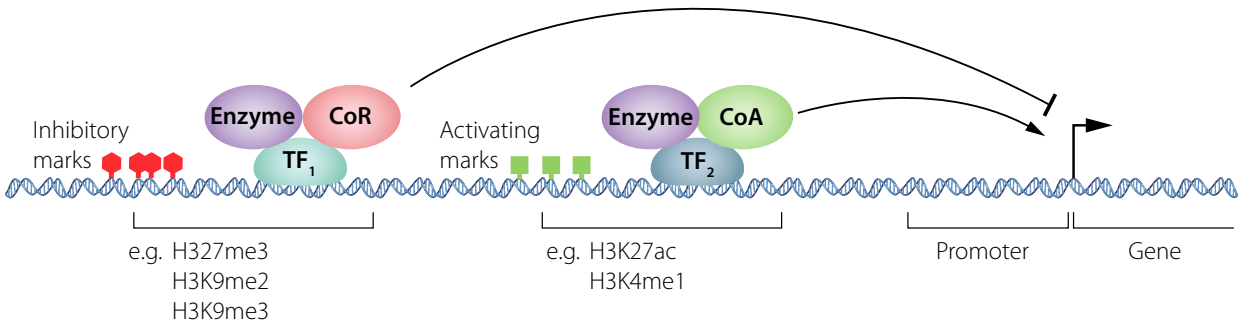


Fig. 2 *Control of gene expression is a complex process involving the interplay of transcription factors (TFs), co-activators (CoAs), corepressors (CoRs) and chromatin-modifying enzymes, which work together to bind enhancers and activate (or repress) the promoters of key genes. See text for more details.*

least in rodents, which may also contribute to the beneficial effect of these agents through the activation of thermogenic pathways [41].

Although most of the antidiabetic actions of TZD drugs are believed to occur within adipocytes, other tissues may also be important. Of interest, the effect of eliminating PPAR-γ in other peripheral insulin-sensitive tissues such as liver and muscle has been far less clear than originally anticipated; for example, tissue-specific ablation of PPAR-γ in the liver does not seem to contribute to systemic insulin sensitivity, but does promote hepatic steatosis [42]. Similarly, loss of PPAR-γ in skeletal muscle results in some insulin resistance but a variable response to TZD administration [43, 44]. Immune cells are also important sites of PPAR-γ action. PPAR-γ ablation in macrophages shifts M1/M2 polarization toward a more inflammatory state and is associated with significant insulin resistance, although rosiglitazone is still effective at reversing the pathology [45–47]. In a more recent study, a subclass of regulatory T cells that expresses PPAR-γ was identified; these cells reside within adipose tissue and when PPAR-γ is eliminated from them, the effect of TZDs on insulin sensitivity is blunted [48]. Findings from other studies have suggested that TZDs may work through activation of PPAR-γ in the central nervous system [49, 50]. Whilst TZDs promote insulin secretion from isolated islets, mice lacking PPAR-γ in β-cells have normal glucose homeostasis and display a normal response to TZDs [51].

PPAR-γ is also modified post-translationally, with significant implications for metabolism. For example, phosphorylation at serine 112 occurs in response to various inducers of cellular stress and insulin resistance; preventing this phosphorylation event preserves insulin sensitivity in animals treated with a high-fat diet [52]. Phosphorylation at serine 273 is also induced by a CDK5/ERK-mediated pathway, and this has profound consequences for the portfolio of target genes bound and activated or repressed by PPAR-γ [53–55]. SUMOylation of PPAR-γ at Lys107 and at Lys365 has also been linked to repression of inflammatory genes and the insulin-sensitizing actions of the hormone FGF21 [56, 57].

The glucocorticoid receptor (GR)

Other NHRs have been implicated in the modulation of insulin sensitivity. The GR is a well-known example, as it mediates insulin resistance in response to both endogenous (as occurs in Cushing's syndrome) and exogenous steroids. Glucocorticoids (GCs), of course, derive their name from their hyperglycaemic actions, which are in contradistinction to their profound anti-inflammatory effects. Several tissues likely contribute to this effect of GCs, including liver, muscle and adipose tissue. For example, GCs have long been known to increase hepatic gluconeogenesis. This is partly mediated by a direct effect of the GR on the expression of the key gluconeogenic genes *Pck1* (encoding PEPCK) and *G6pc* (encoding G6Pase), such that roughly 50% of mice lacking the GR in the liver die as a result of hypoglycaemia [58, 59]. Additionally, pharmacological inhibition of the GR reduces PEPCK and G6Pase and improves glucose tolerance [60]. GCs also increase gluconeogenesis indirectly, via enhanced proteolysis in muscle, thus increasing substrate availability for glucose production. The GR also promotes hepatic steatosis in part by repressing hydrolysis and oxidation of triglyceride, which may itself cause insulin resistance (although the arrow of causality here is controversial) [61, 62]. Finally, central effects of the GR have to be considered as well, as injection of dexamethasone into the arcuate nucleus of the hypothalamus also causes hepatic insulin resistance, an effect which does not require the increased appetite and weight gain often encountered with GC administration [63].

In muscle, GR levels correlate with the overall insulin sensitivity of diabetic patients [64]. The GR has been shown to directly bind to regions regulating genes involved in insulin signalling, which presumably contributes to the reduced insulin-stimulated glucose uptake seen after GC treatment of myocytes [65]. This may also contribute to GC-induced proteolysis and inhibition of protein synthesis in muscle tissue, although the latter also involves additional pathways including repression of the insulin signalling mediator TORC1 by *Ddit4* (which encodes the protein REDD1), a gene upregulated by GCs and the GR [66]. Mice lacking *Ddit4* are protected from GC-induced muscle wasting [67].

GCs and GRs are well known to cause insulin resistance in cultured adipocytes [6, 9], although a definitive role for the adipose GR *in vivo* has not been clearly demonstrated. This effect is due in part to a GC-mediated increase in oxidative stress and also relies on the induction of target genes

such as *Vdr*, *Colq* and *Tmem176a* with no obvious relationship to insulin signalling or action [10]. Interestingly, the GR has also been shown to mediate the insulin resistance caused by the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) in adipocytes [10].

In the unliganded state, the GR is normally sequestered in the cytosol bound to heat shock proteins and various other chaperones; GC binding causes release of the receptor and translocation to the nucleus. Once in the nucleus, the GR can influence gene expression in several different ways. It can bind directly to glucocorticoid response elements (GREs) in promoters and enhancers of target genes. This has been thought to involve homodimerization of the GR, although the GR forms monomers or heterodimers with other transcription factors [59]. Alternatively, the GR can bind to DNA indirectly by 'tethering' through a different transcription factor. In general, it has been believed that the anti-inflammatory effects of GCs involve this tethering action of the GR, specifically through interaction with and inhibition of pro-inflammatory transcription factors such as AP-1 and NF-κB, whilst the negative side effects of GCs (e.g. insulin resistance) have been proposed to occur through direct binding to DNA. As mentioned above, there is definitely a role for direct binding of the GR to gluconeogenic genes such as *Pck1* and *G6pc*, but it is worth noting that mice bearing a mutant form of the GR that cannot homodimerize, but is fully able to tether to other factors, the receptor still mediates insulin resistance in response to GCs [68]. There may also be a role for rapid actions of the GR in insulin signalling that do not involve DNA binding at all, either direct or indirect [69].

Fox01

Another transcription factor that plays a major role in insulin signalling is FoxO1, one of four mammalian FoxO factors with overlapping functions (the others being FoxO3a, FoxO4 and FoxO6). FoxO1 shuttles between the nucleus and cytosol in an insulin-dependent fashion, with Akt-mediated phosphorylation causing nuclear exclusion and subsequent degradation. Combinatorial knockout studies in mice suggest that FoxO1, FoxO3a and FoxO4 act cooperatively in the liver to promote gluconeogenesis, and there is evidence that FoxO6 contributes to hepatic control of glycaemia as well [70, 71]. In adipose tissue, FoxO1 inhibits adipogenesis, whilst in mature cells, it promotes the expression of UCP-1 and thermogenesis, with a subsequent beneficial effect on adiposity and insulin sensitivity [72]. This is the precise opposite of its role in $AgRP^{+}$ hypothalamic neurons, where *loss* of FoxO1 reduces food intake, body weight and hepatic glucose production [73]. In liver, the specific gene targets of FoxO1 include *Pepck* and *G6pc*, which encode the two key gluconeogenic enzymes; metabolically relevant gene targets in other tissues are more obscure.

FoxO1 is highly modified post-translationally, with profound effects on its activity. In addition to phosphorylation by insulin/Akt as mentioned above, other sites are phosphorylated in response to oxidative stress via JNK1. This phosphorylation promotes FoxO1 activity on a subset of antioxidant genes, in contrast to insulin-induced phosphorylation which inactivates FoxO1. AMP kinase (AMPK) can also phosphorylate FoxO1, which inhibits hepatic glucose production [74]. Other post-translational modifications of FoxO1 include acetylation, which has context-dependent effects on activity, and O-GlcNAcylation, which promotes FoxO1 activity and gluconeogenesis [75, 76].

There are many other transcription factors with major effects on local and systemic insulin sensitivity. LXRα, for example, promotes insulin sensitivity in white adipose tissue (by enhancing Glut4 expression) and in liver (by suppressing gluconeogenesis) [77]. ChREBP is also a mediator of insulin action in both white adipose tissue and liver [78, 79]. Other transcription factors are more complex; vitamin D receptor (VDR) inhibits glucose uptake in cultured adipocytes in a ligand-independent way, whilst also causing increased macrophage M2 polarization, which increases insulin sensitivity [10, 80]. There is evidence from numerous epidemiological studies suggesting that vitamin D promotes insulin sensitivity; of note, direct administration of vitamin D to human subjects has improved insulin action in some studies but not others [81, 82].

Regulation at the level of the transcriptional cofactor

Hundreds of proteins have now been identified as transcriptional co-activators and corepressors. As mentioned previously, these proteins do not bind DNA themselves but act by forming a complex with transcription factors and regulating their activity. Many cofactors are highly responsive to nutritional conditions, which they sense through post-translational modifications such as

phosphorylation and acetylation. These modifications then cause the cofactor to associate with or disassociate from its cognate factor, thus changing both the magnitude of the transcriptional effect and the specific target gene program. One might anticipate that the effects of many cofactors on insulin sensitivity could be predicted from their affiliation to specific transcription factors. Thus, PPAR-γ co-activators might be predicted to enhance insulin sensitivity, whereas corepressors should have the opposite effect. In many cases, this is what is seen; for example, loss of the PPAR-γ corepressor NCoR1 in adipose tissue of NCoR1 knockout mice causes insulin sensitization and enhanced glucose tolerance [83]. It is interesting that TZDs fail to further enhance insulin action in these animals, suggesting that a major role for PPAR-γ agonists is to remove the negative influence of NCoR1 in fat. It is not always easy to predict how a given cofactor will affect metabolism, however, given that many cofactors bind to and influence the actions of a multitude of transcription factors. Thus, the net effect of gaining or losing such a cofactor may reflect the integrated response of different factors in different tissues under different physiological conditions. For example, loss of the PPAR-γ co-activators NCoA2 or NCoA3 actually improves insulin sensitivity, primarily as a result of increased browning and resistance to diet-induced obesity [84, 85].

Another example of a group of metabolic co-activators is a family of cAMP responsive element-binding protein (CREB) co-activators called the cAMP-regulated transcriptional co-activators [(CRTCs) comprising CRTC1–3]. CRTCs operate in a variety of metabolic tissues, the best studied of which is the liver, where CRTC2 enhances the CREB-dependent regulation of key gluconeogenic genes such as *Pepck1* and *G6pc*. Loss of either CTRC2 or CREB greatly reduces hepatic glucose production, improves insulin signalling and sensitivity and can result in hypoglycaemia [86–89]. CRTC3, on the other hand, affects catecholamine action in adipose tissue by inducing the negative regulator Rgs2. Thus, CRTC3 null mice are lean and insulin sensitive, with enhanced insulin and catecholamine signalling. Interestingly, a human gain-of-function CRTC3 variant is associated with obesity in Mexican Americans [90].

Other examples of nutritionally regulated transcriptional cofactors include the PGC-1 family of proteins. PGC-1α plays multiple roles in insulin sensitivity. In muscle, it co-activates MEF2C and increases Glut4 expression, thus enhancing insulin action [91]. In liver, however, PGC-1α binds to FoxO1 and the GR, and is a critical component of the gluconeogenesis response to fasting, with strong effects to increase glycaemia [92]. In brown adipose tissue, meanwhile, PGC-1α is a dominant driver of thermogenesis, mitochondrial biogenesis and fatty acid oxidation, effects which improve insulin sensitivity and are mediated by the transcription factors IRF4, ERR-α and PPAR-α [92–94]. PGC-1β, on the other hand, has been implicated as a driver of insulin resistance in response to fructose ingestion [95].

Regulation at the level of the chromatin-modifying enzyme

Most of the enzymes that control the chromatin state, including the various DNA methylases and demethylases, along with the various histone-modifying enzymes, are broadly expressed in multiple cell types. One might therefore reasonably expect that influencing the activity of these factors might cause a wide variety of pathologies in many different systems; in fact, the reliance on these enzymes for normal development might predict that embryonic lethality would ensue. Whilst this is true for some of these factors, many others show highly selective defects when targeted, including metabolic problems. One such example is Jhdm2a, which encodes a histone 3 lysine 9 (H3K9) demethylase. Targeted ablation of this enzyme results in mice with obesity, hyperlipidaemia and insulin resistance. This is at least in part due to repression of PPAR-α and UCP-1 expression in adipocytes [19, 21]. Similarly, the *de novo* DNA methyltransferase DNMT1 represses expression of adiponectin in fat; pharmacological inhibition of DNMTs relieves this and improves insulin sensitivity in obese mice [96].

It should be noted that the distinction between some chromatin-modifying enzymes and transcriptional cofactors is blurred. For example, the well-studied co-activators p300 and CREB (cyclic AMP response element-binding protein) binding protein (CBP) have intrinsic HAT activity. These factors also interact with PCAF, another HAT protein. It should also be noted that histones are not the only targets of these enzymes that may be relevant for insulin action. PCAF, for example, acetylates PGC-1α in the liver, thus repressing its gluconeogenic actions [97]. In addition, one cannot assume that the enzymatic activity of a chromatin-

modifying protein is required for its metabolic functions. For example, mice lacking HDAC3 in liver have profound hepatosteatosis with enhanced insulin sensitivity; repletion of a catalytically dead HDAC3 improves the lipid phenotype, whereas a different allele that cannot interact with corepressors such as SMRT and NCoR does not [98, 99]. There is obviously much to be learned about how these general factors exert their actions on metabolism.

Integrating the actions of transcription factors, cofactors and chromatin-modifying enzymes: an example

Although the effects of transcription factors, transcriptional cofactors and chromatin-modifying enzymes have been presented here as separable control elements, they all operate in tandem to coordinate metabolic gene expression. To illustrate this, the transcriptional response to fasting and feeding and its effects on hepatic gluconeogenesis can be considered (Fig. 3). Key gluconeogenic genes, such as *Pepck1* and *G6pc*, are induced by a variety of transcription factors, including FOXO1 and CREB. FOXO1 is translocated out of the nucleus in response to insulin, whilst CREB is activated by glucagon signalling. There is a major role also for cofactors, as the CRTC co-activators are also transposed by feeding status, moving into the nucleus in response to fasting (glucagon) and shuttling out when insulin is high. FOXO1 is in turn activated by the chromatin-modifying

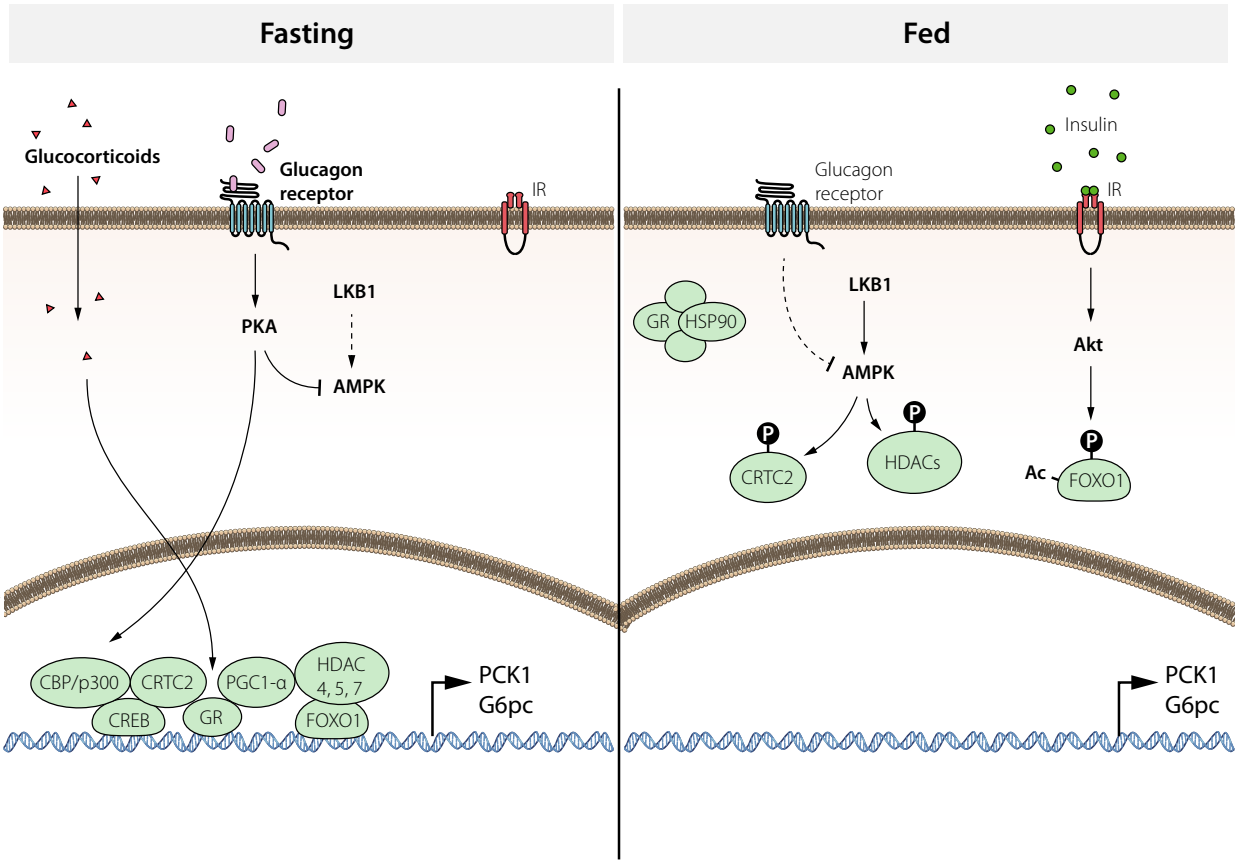


Fig. 3 *The transcriptional response to fasting and feeding in the liver is illustrative of changes that occur during the development of insulin resistance and type 2 diabetes. Hormonal signals such as insulin, glucagon and glucocorticoids are transduced into transcriptional responses via the actions of key transcription factors such as the glucocorticoid receptor (GR), FoxO1 and CREB. The activity of these transcription factors is modulated by many cofactors, such as CRTC2, CBP/p300, PGC-1α and various histone deacetylases (HDACs), whose activity is regulated by a variety of post-translational modifications. See text for further details. IR, insulin receptor; AMPK, 5′ AMP-activated protein kinase; HSP90, heat shock protein 90; LKB1, liver kinase B1; AKT; protein kinase B.*

enzymes HDAC4, HDAC5 and HDAC7, which move into and out of the nucleus under the control of glucagon and AMPK, respectively [100]. Superimposed on these elements are additional levels of control provided by the co-activator/chromatin-modifying enzymes p300 and CBP, which acetylate a key lysine residue (K628) on CRTC2 in response to glucagon. This stabilizes the CRTC2 protein and enhances its hyperglycaemic effects [101]. CRTC2 is further regulated by additional layers of acetylation and deacetylation, phosphorylation and glycosylation in response to nutritional cues [35]. Taken together, there is a finely honed suite of regulatory events that coordinate hepatic insulin sensitivity in a robust and tunable way.

Variation in transcription factor binding as a source of differences in human insulin sensitivity

Many genetic studies have been performed to identify variants that associate with obesity, T2D and additional glycaemic traits (e.g. fasting insulin and fasting glucose). Amongst the hundreds of loci identified to date are several that lie in or near a transcription factor. Most of these variants are in noncoding regions, but several change coding sequence. One of the best known of these is in the *PPARG* locus, changing a proline to an alanine at position 12. Interestingly, the rarer alanine variant has been proposed to be protective against T2D [102]. This relationship was recently called into question, however, by the demonstration that the P12A coding variant may not be the causal allele; rather, a noncoding polymorphism in linkage disequilibrium with P12A seems to affect binding of the transcription factor PRRX1 to the *PPARG* promoter, which reduces PPAR-γ levels and has negative effects on glycaemia and triglyceridaemia [103]. T2D and insulin action have also been linked to noncoding variants near the gene encoding the transcription factor KLF14, which may act as a regulator of adipose gene expression [104], although formal proof is still lacking that altered KLF14 levels or activity are responsible for the increased disease risk. Recently, a coding polymorphism in the human *TP53* gene, encoding the p53 tumour-suppressing transcription factor, was identified with associations with T2D and obesity [105, 106]. This P72R variant causes obesity and impaired insulin action when knocked into a mouse model [107].

These examples emerged from studies of common variants, but much genetic variation occurs too infrequently to be captured by a genome-wide association study (GWAS). Some of these rare variants are certain drivers of insulin resistance and diabetes risk. For example, sequencing the *PPARG* gene of almost 20 000 individuals led to the discovery of 49 previously unannotated coding missense variants of the *PPARG* gene with a minor allele frequency of <0.5%. Nine of these variants were found to have a deleterious effect in a high-throughput adipogenesis assay, and these were also associated with a significantly higher risk of T2D [108]. As large amounts of exome sequencing data accumulate, even more rare variants in transcription factors will be associated with metabolic traits. The authors of a recent study of 64 706 exomes from individuals of diverse ancestry identified 53 384 polymorphisms in the DNA-binding domains of a curated set of over 1200 human transcription factors [109]. They also identified 4552 unique nonsense mutations resulting in partial or full truncation of the DNA-binding domains of these factors, but did not link any of this potentially deleterious variation to metabolic traits such as insulin sensitivity; this will certainly occur as deeper phenotyping of human subjects catches up with the rich genetic databases. Furthermore, we need to keep in mind that this does not include polymorphisms in transcriptional cofactors or in chromatin-modifying enzymes, which will likely emerge as important sources of metabolic variation in the future.

As stated above, most genetic variation occurs outside of coding regions, with a huge number of polymorphisms that affect *cis*-regulatory elements such as promoters and enhancers. Such noncoding SNPs alter the binding of transcription factors and can have a major impact on metabolic traits and disease risk. For example, noncoding variants that differ between mouse strains have been identified within PPAR-γ motifs [110]. The strength of PPAR-γ binding at these loci depends on the specific sequence at that site, as does the response to TZD drugs. Furthermore, analogous polymorphisms have been identified in human subjects that appear to underlie metabolic traits such as high density lipoprotein (HDL) and triglyceride levels [110].

Opportunities for therapeutic intervention

How will learning about transcriptional regulation of insulin sensitivity help in the battle against metabolic disease? In the case of NHRs, the

connection is obvious; PPAR-γ agonist TZD drugs are already marketed for insulin resistance in T2D and polycystic ovarian syndrome [38]. The use of these agents has diminished somewhat over time, in part because of side effects including weight gain, increased plasma volume and bone loss, but also because of reports that TZD use may predispose to congestive heart failure [17]. Many of these concerns now appear to have been overblown, and additional pharmaceutical development in the PPAR-γ agonist field may be seen in the coming years. For example, ligands have been identified that prevent phosphorylation of Ser273 of PPAR-γ; these agents promote insulin sensitization in a mouse model of obesity without causing weight gain or haemodilution [54]. Similar agents that act on other NHRs may also be useful, including specific GR modulators or vitamin D analogues. Other, non-nuclear receptor transcription factors are not considered to be quite as 'druggable', but their upstream pathways may yet prove to be exploitable for therapeutic benefit. Similarly, most nuclear cofactors are not traditionally thought of as amenable to small-molecule therapy, but this viewpoint may be changing, as recent studies have identified small-molecule inhibitors of the co-activators NCoA1 and NCoA3 [111].

Chromatin-modifying enzymes, however, have been successfully targeted with drugs numerous times, and new compounds are continually being identified and tested for clinical utility [33, 34]. This area of investigation has moved most rapidly in the cancer arena, with multiple chemical epigenomic modifiers already undergoing extensive clinical testing. Such drugs have not been tested specifically for insulin resistance in humans, but there are preclinical studies that provide encouragement. For example, several nonspecific HDAC inhibitors improve metabolic function in rodent models of obesity and insulin resistance, including several derivatives of sodium butyrate and trichostatin A [112–114]. More specific drugs have also been tested and inhibitors of class I HDACs (HDACs1–3, 8 and 10) enhanced mitochondrial activity in muscle and fat and increased insulin sensitivity in obese mice [115]. Short hairpin-induced repression of some class II HDACs (HDACs 4, 5 and 7) diminished hepatic gluconeogenesis, an effect also seen with small-molecule class II HDAC inhibitors [100]. One important caveat in the interpretation of these studies is that many HDACs act on nonhistone proteins, which may mediate some of the beneficial metabolic actions of these drugs.

As mentioned above, chemical DNA methyltransferase inhibition using the drug RG-108 has also proven beneficial in obese, diabetic mice, causing an improvement in insulin sensitivity that was linked to an increase in adiponectin gene expression [96]. This is a burgeoning area of pharmaceutical investigation, and additional agents should become available in a relatively short period of time.

Summary and future directions

Insulin resistance has been classically portrayed as a fundamental problem of insulin signalling, with an almost exclusive focus on post-translational regulatory mechanisms. It seems clear, however, that transcriptional activity is altered in multiple tissues and cell types that have direct bearing on systemic insulin action and that these processes are beginning to be unravelled as new technologies appear that make the study of such mechanisms tractable on a genome-wide scale. It can be anticipated that future developments in antidiabetic drug therapy will focus on these pathways, as we strive to triumph in the ongoing battle against metabolic dysfunction.

Conflict of interest statement

The author receives research funding from Merck Laboratories.

Acknowledgements

The author is supported by NIH grants R01 DK 085171, R01 DK 102173 and R01 DK 102170.

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