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Review

Role of PPARg2 transcription factor in thiazolidinedione-induced insulin sensitization

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Abstract

Objectives Adipose tissue is the key regulator of energy balance, playing an active role in lipid storage and metabolism and may be a dynamic buffer to control fatty acid flux. Peroxisome proliferator-activated receptor gamma isoform-2 (PPARg2), an isoform of the nuclear hormone receptor superfamily, has been implicated in almost all aspects of human metabolic alterations such as obesity, insulin resistance, type-2 diabetes and dyslipidaemia. The PPARg2 isoform is highly present in adipose tissue where it functions as a thrifty phenotype, which promotes adipocyte differentiation and triglyceride storage. Thiazolidinediones, antidiabetic drugs, induce insulin sensitivity by controlling adipokines. The thiazolidinediones bind with PPARg2 in adipocytes and exert an agonist effect by enhancing adipogenesis and fatty acid uptake. Thiazolidinediones stimulate PPARg2, by which they down-regulate tumour necrosis factor-α, leptin, interleukin-6 and plasminogen and also enhance insulin sensitivity. The aim of this work is to define role of PPARg2 transcription factor in thiazolidinedione-induced insulin sensitization.

Key findings The PPARg2 alters the transcription of the target gene. This altered gene transcription results in the up-regulation of insulin-sensitizing factors and down-regulation of insulin-resistant factors. The variant *Pro12Ala* of the *PPARg2* gene is an important modulator in metabolic control in the body. Thiazolidinediones stimulate PPARg2 transcription factor by which PPARg2 binds to responsive elements located in the promoter regions of many genes and modulates their transcriptive activity. There is a strong mutual relationship between receptor binding and agonism, which is evidence of the insulin-sensitizing target of thiazolidinediones in *PPARg2*. This evidently increases the biological potency of the glucose-lowering effect of thiazolidinediones *in vivo* as well as their antidiabetic activity.

Conclusions PPARg2 transcription factor plays an important role in treatment of type-2 diabetes with thiazolidindiones. The variant *Pro12Ala* of the *PPARg2* gene promotes the activity of thiazolidinediones in minimizing insulin resistance. Transcriptional activity of *Pro12Ala* variant improves the activity of insulin. Thus thiazolidinediones promote the phosphorylation of PPARg2 to induce insulin sensitivity.

Introduction

Adipose tissue is largely regarded as a depot for fuel storage in the form of triglyceride. It is an essential, highly active metabolic and endocrine organ.[1] It plays an important part in lipid storage with multiple individual deposits in subcutaneous, intra-abdominal and intra-thoracic regions.[2,3] The important endocrine function of adipose tissue is emphasized by the adverse metabolic consequences of both adipose tissue excess and deficiency. Its excess, or obesity, particularly in the visceral compartment, is associated with insulin resistance, hyperglycaemia, dyslipidaemia, hypertension and prothrombotic and pro-inflammatory states and its deficiency, or lipodystrophy, is also associated with features of the metabolic syndrome in both humans and rodents.[1] Adipose tissue is also responsible for the secretion of a variety of proteins such as tumor necrosis factor (TNF)-α, adipsin, plasminogen activator inhibitor-1, leptin, resistin and adiponectin. These

proteins have similar structural properties to cytokines, have been collectively called adipocytokines and are implicated in a wide range of biological effects. Dysregulation of adipocytokines in obesity causes development of insulin resistance and vascular disorders.[4]

Pathogenesis of Insulin Resistance

Adipose tissue plays an important role as an endocrine organ that secretes several polypeptides which are responsible for the regulation of metabolism, including insulin sensitizers such as adiponectin and insulin resistance factor such as resistin and TNF-α.[1,5] In obesity, a cytokine, TNF-α, is overproduced by adipose tissue and causes systemic insulin resistance by interfering with the insulin-signalling cascade. Impaired adipose tissue function leads to decreased triglyceride uptake and also increased plasma fatty acid concentration, which leads to the development of insulin resistance in muscle, fatty liver and dyslipidaemia. Adiponectin circulates in adipose tissue at relatively high concentration (μg per ml) and shares sequence homology with human type VIII and X collagen, complement factor C1q and TNF-α. In rodents, this form of protein is called ACRP30. Adiponectin/ACRP30 promotes weight loss, free fatty acid oxidation and decreased plasma glucose levels in mice. Its level tends to be higher in a state of insulin sensitivity and decreased in a state of insulin resistance.[4,6,7] A central regulator of adiposity is leptin, which affects glucose homoeostasis. Resistin is a novel adipose-specific cysteine-rich protein, which has recently been developed and is responsible for impairment of insulin sensitivity and glucose tolerance.[7] Insulin resistance is associated with obesity, diabetes, hypertension, dyslipidaemia and atherosclerosis.[8] Lipolysis and lipid metabolism is dysregulated by high-fat diet (HFD). Eight weeks of HFD feeding increased adipose triglyceride lipase (ATGL) content but reduced hormone-sensitive lipase (HSL) phosphorylation and perilipin content severely.[9] HSL is the enzyme primarily responsible for the hydrolysis of triacylglycerols and diacylglycerols, which releases free fatty acids (FFAs) in male mice. ATGL/desnutrin is a newly identified lipase in human that predominantly performs the initial step in triglyceride hydrolysis and over-expression of ATGL increases lipolysis in adipocytes.[10] A HFD powerfully inhibited forskolin-induced AMP kinase (AMPK) activation as well as peroxisome proliferator-activated receptor gamma (PPARg) coactivator-1α expression in fat depots. Lipolysis in the white adipose tissue (WAT) of humans and rodents is regulated by different lipases, such as HSL, ATGL, monoacylglycerol lipase (MAGL) and adipose-specific phospholipase A2 (AdPLA). AdPLA belongs to a new group of intracellular calcium-dependent PLA2. It is highly expressed during pre-adipocyte differentiation into adipocytes. It increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency.[11] In the state of insulin resistance, insulin receptor substrate (IRS) phosphorylation, PI 3-kinase activity and glucose transporter-4α (GLUT-4α) activity are impaired.[12] Elevated FFAs and TNF-α and levels of several other proteins are expressed in fat tissue (e.g. Figure 1). These factors are responsible for the genesis of obesity-related insulin resistance.[13] Moreover, it has been shown that FFA, rather than TNF-α, is mainly responsible for development of insulin resistance, whereas in the later stage of the disease, both TNF-α and FFA play an important role.[8,14] This results in the increment of fatty acid concentrations in serum and also in non-adipose tissues like liver and muscle.[1,5]

Physiology of Peroxisome Proliferator-Activated Receptor Gamma Isoform-2

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belongs to the nuclear hormone receptor superfamily. These receptors are mostly found in adipose tissue and are also expressed in skeletal muscle and macrophages, as well as other tissues.[4,15] The *PPARg* gene is located on chromosome 3p25 (OMIM number 601487). The *PPARg* gene contains nine exons, spans more than 100 kilobases and produces two protein isoforms, peroxisome proliferator-activated receptor gamma isoform-1 (PPARg1) and peroxisome proliferator-activated receptor gamma isoform-2 (PPARg2) by alternative mRNA splicing. *PPARg1* is encoded by 8 exons, using exons 1–6, A1, and A2, but *PPARg2* is encoded using exons 1–6 and B.[16] An isoform of PPAR, such as PPARg2, can alter the transcription of the target gene. Expression of each PPARg2 isoform is tissue dependent.[17,18] An alteration of gene transcription results in the up-regulation of the insulin-sensitizing factor and down-regulation of the insulin-resistant factor. PPARg2 is present predominantly in adipose tissue and large intestine, intermediately in the kidney, liver and small intestine and to a limited extent in muscle.[8,13] There are different types of target gene for *PPARg2* transcription. In differentiated cells and tissues, TNF-α and leptin expression is reduced by *PPARg2* activation. Two genes of fatty acid metabolism, lipoprotein lipase and the fatty acid binding protein aP2, appear to be a direct target of *PPARg2* activation.[19–22]

Classification and Structure

PPARs have been categorised as second class nuclear receptors. The PPARg receptor is composed of six structural regions (A–F) in four functional domains. The second class of nuclear receptor shares some properties such as heterodimerizing with retinoid X receptor (RXR) and binding to direct repeat sequence of nucleotides. The phosphorylation of a domain

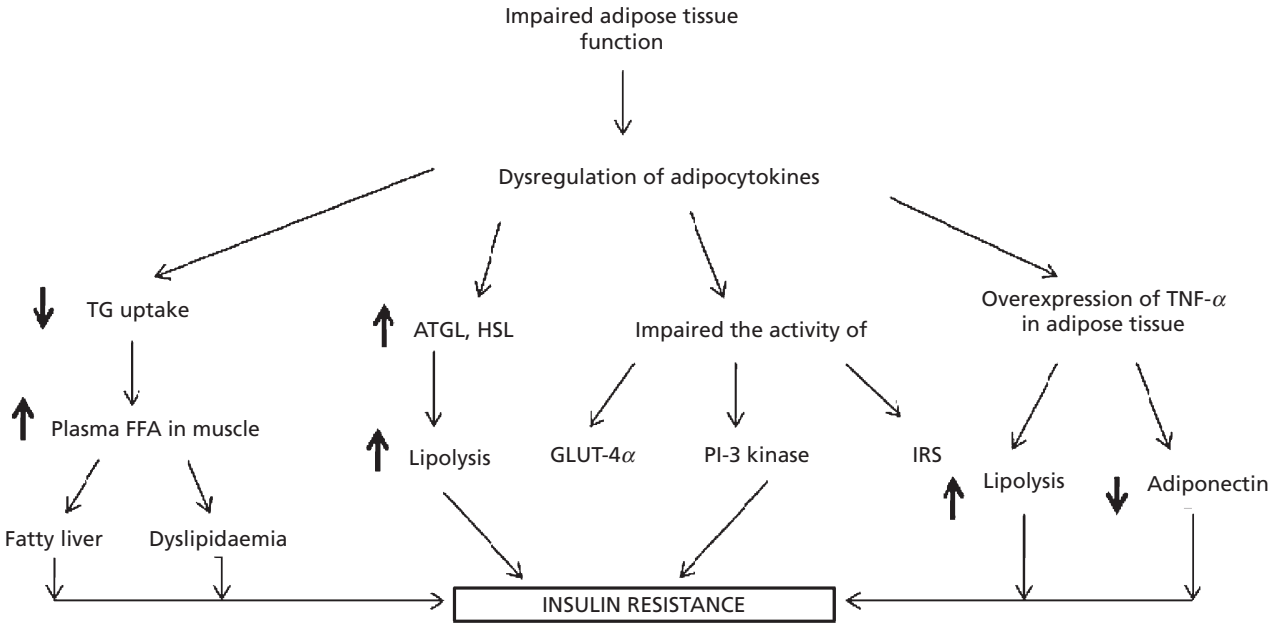

Figure 1 Pathogenesis of insulin resistance. IRS, insulin receptor substrate; GLUT-4α, glucose transporter-4α; PI3-kinase, phosphoinositide 3-kinase; TG, triglyceride; FFA, free fatty acid; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase.

modifies it covalently and changes the ligand-binding affinity of the receptor.[23] *PPARg2* transcription in humans produces a distinct protein. This protein contains an additional 28 amino acids at the N-terminal.[10] The C-region holds the DNA-binding domain that is responsible for targeting of PPARs to specific sequence of nucleotide within the regulatory region of responsive genes.[24] Another important domain, the activation function-2 domain is adjacent to C terminals, agonist binding to this domain will change its conformation. An active conformation subsequently results in an increase in the activity of this receptor.[8,25] There are different types of genetic variants that are present in the *PPARg2* gene[26] (e.g. Figure 2). These include (1) very rare gain-of-function mutation and (2) loss-of-function mutation variants. *Pro115Gln* is a gain-of-function mutation variant, which is associated with obesity but not insulin resistance.[27] *Val290Met* and *Pro467Leu* are loss-of-function mutation variants found in individuals with very severe insulin resistance but a normal body weight. *Pro12Ala* is a very common variant with reduced function.[26,28]

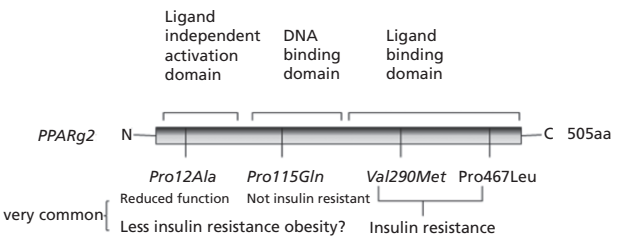

Figure 2 Functional domains and variants of *PPARg2* gene.[26] The *PPARg2* gene has different domains and variants. The variant *Pro12Ala* is very common and has a reduced insulin resistance property. A very rare *Pro115Gln* is a gain-of-function mutation variant that associated with obesity but not insulin resistance and two *Val290Met and Pro467Leu* variants are loss-of-function mutations that are found in individuals who have severe insulin resistance but normal body weight. *PPARg2*, peroxisome proliferator-activated receptor gamma isoform-2.

PPARg2 Transcription Factor

PPARg2 belongs to family of nuclear receptors, which includes 48 human transcription factors. These factors are regulated by direct binding of steroids, thyroid hormones, vitamins, xenobiotics and lipid metabolites. PPARg2 plays a critical role in the regulation of lipid metabolism genes.[5,29] The Ala allele is a *Pro12Ala* polymorphism of isoform PPARg2 which reduced risk for type 2 diabetes (T2DM), reduced weight gain and improved insulin sensitivity.[20–22] Gene-environment interaction has a primary role in the effect of the *Pro12Ala* variant (*rs1801282*) of *PPARg2* on adiposity, plasma lipid and insulin sensitivity by eliminating high-fat feeding. The variant *Pro12ala* of the *PPARg2* gene is an important modulator in metabolic control in the body.[2,30,31] The *PPARg* transcription factor combines with the retinoid X receptor, 'RXR', to form a heterodimer that is responsible for regulation of various genes involved in lipid and glucose metabolism, fatty acid transport, adipocyte

differentiation, carcinogenesis and inflammation.[32–34] The *PPARg2* heterozygote enhances insulin sensitivity.[15] The functions of PPARg are affected by two protein isomers. These isomers are derived from the same gene by alternative promoter usage and splicing. The PPARg2 isoform is highly present in adipose tissue where it functions as a thrifty phenotype which promotes adipocyte differentiation and triglyceride storage.[5,35–37] PPARg2 isoforms play an important role in regulating the development and metabolism of adipocytes. The role of transcription factor PPARg2 has been recently identified. *PPARg2* transcription factor regulates not only lipid metabolism but also secretory proteins, like leptin and TNF-α, which influence skeletal muscle insulin sensitivity.[8,26] cAMP response element binding proteins (CREBPs) also regulate lipid metabolism by enhancing lipid accumulation. They activate *PPARg2* transcription in HepG2 cells. Two critical CREBPD-binding occur on the −324/−311 and −158/−145 of humans, which regulate and promote the function of the *PPARg2* gene.[15,38,39] The natural ligands of PPARg include fatty acids,[40] components of oxidized low-density lipoproteins[41] and alkyl phospholipids, which include lysophosphatidic acid[42] and nitrolinoleic acids.[43] PPARg binds with an agonist ligand to produce conformational change that attract transcriptional co-activators such as the steroid receptor co-activator family (SRC). When the ligand is not present, PPARg has the capability of activating the silence genes. With these genes PPARg is bound by recruiting transcriptional co-repressor complexes. It contains nuclear receptor co-repressor or SMRT (silencing mediator of retinoid and thyroid receptors).[5,44–46] The transcriptional co-activators and co-repressor are present in multiprotein complexes, which are exhibited in histone-modifying enzymes like histone acetyltransferases and histone deacetylases. The activity of these histone-modifying enzymes regulates gene transcription by altering chromatin structure. Another factor that can cooperate with *PPARg2* is adipocyte determination and differentiation factor1/sterol response element binding protein 1 (ADD1/SREBP1). These factors acts as a regulatory key in cholesterol homoeostasis and fatty acid metabolism. ADD1/SREBP1 increases the transcriptional activity of *PPARg* by coexpression of it with *PPARg*, even without adding a *PPARg* ligand. *PPARg2* regulates genes that are involved in metabolism by transcription mechanism.[5] The *PPARg2* mutation results in the alanine substitution for proline at codon 12 of the *PPARg*2 gene. The frequency of the 12Ala allele has been found to range from 2% to 18% in healthy humans.[32,47] The *Pro12Ala* polymorphism of the *PPARg2* gene is associated with type-2 diabetes mellitus and peripheral insulin sensitivity in a population with a high intake of oleic acid. It also involves synthesis of fatty acid, which is an important substrate for energy metabolism. The biosynthesis of fatty acids is catalysed by fatty-acid synthase (FAS) and acetyl-CoA carboxylase (ACC). These are enzymes of lipogenesis that play an important role in the weight variability of abdominal adipose tissue.[4] The interaction between monounsaturated fatty acids (MUFA) and the Ala-12 allele of *PPARg2* is also important in type-2 diabetes mellitus. The contribution of MUFA to the variance of the homoeostasis model assessment insulin resistance index (HOMA IR) is by two-way interaction between the intake of MUFA and the *Pro12Ala* polymorphism of *PPARg2*. This interaction lowers the risk for type-2 diabetes due to an association between the *Pro12Ala* polymorphism of *PPARg2* and dietary MUFA. Obese subjects have the Ala-12 allele and also have higher HOMA IR values, especially if their consumption of MUFA was low.[48]

PPARg2 Influences Insulin Sensitization

PPARg2 activation influences adipose differentiation and proliferation of pre-adipocytes into mature fat cells (particularly peripheral or subcutaneous). The uptake of fatty acids is facilitated by up-regulation of transporter in adipocytes (e.g. lipoprotein lipase transporter, fatty-acid transporter 1 and glycerol kinase). Fat cells originate from a fibroblast-like preadipocyte and develop into a mature, lipid-enriched adipocyte.[5,49] The highest PPARg2 is present in adipose tissue and the activation of PPARg2 transcription factor induces adipogenesis.[50] Two PPARg isoforms are developed by differential promoter usage and splicing. These two isoforms are g1 and g2. Thirty additional amino acids are present in the N-terminal end of PPARg2. PPARg1 is also found in macrophages, colon epithelia and endothelium.[51–53] PPARg2 is associated with reduced transcriptional activity *in vitro* and enhanced insulin sensitivity in humans *in vivo* in adipose tissue. PPARg2 has many influences, affecting adipocyte biology, insulin action, cardiovascular disease, inflammation, renal function and tumour biology.[5,26] PPARg2 is associated with several phenotypes such as insulin resistance, partial lipodystrophy, type 2 diabetes and hypertension.[54] PPARg2 is also expressed in liver and induced acute hepatic steatosis while markedly decreasing peripheral adiposity. These changes are accompanied by increased energy expenditure and improved systemic insulin sensitivity. Hepatic vagotomy and selective afferent blockage of the hepatic vagus revealed that the effects on peripheral tissues involve the afferent vagal nerve. PPARg2 may function to protect agonist metabolic perturbation induced by excessive energy storage in liver.[55] PPARg2 is highly expressed as a target of antidiabetic thiazolidinedione drugs that reverse insulin resistance but they promote weight gain. The phosphorylation of PPARg2 reduces its activity *in vitro* but this phosphorylation is responsible for insulin sensitivity *in vivo*. Some partial agonists, such as FMOC-L-leucine,[5] prevent PPARg2 phosphorylation or induce the conformation of

non-phosphorylated PPARg2 to enhance insulin sensitivity without increasing body weight.[56] PPARg2 activity is regulated by MAP kinase phosphorylation of serine 112, which is responsible for reducing the transcription activity of *PPARg2*.[57,58] The transcription activity of *PPARg2* is significant as various growth factor and cytokines. These factors are also affect the transcriptional activity of several genes that are responsible for lipid metabolism.[56]

Thiazolidinediones

Thiazolidinediones are antidiabetic agents that are agonists for the PPARg2 nuclear receptor. Adipogenesis is mostly regulated by PPARg2 *in vitro* and *in vivo*. Thazolidinediones, ligands for PPARg2, effectively ameliorate insulin resistance and are used clinically to improve type-2 diabetes and in the treatment of insulin-resistant states.[4,8,56,59,60] Ciglitazone, troglitazone, rosiglitazone and pioglitazone are some drugs of this group (e.g. Figure 3). The effect of thiazolidinedione treatment is based on modulating the circulating adiponectin levels in lean and obese diabetic patients. It increases adiponectin levels in patients with impaired glucose tolerance and type-2 diabetes. The thiazolidindiones can enhance the expression and secretion of adiponectin through the activation of its promoter and also antagonize the suppressive effect of TNF-α on the production of adiponectin. Therefore, thiazolidinediones might prevent atherosclerosis in diabetic patients with insulin resistance.[4,7] Troglitazone, the first thiazolidinedione marketed as an antidiabetic agent, was withdrawn from the market due to liver toxicity in several patients.[62] The use of rosiglitazone was also banned by US Food and Drug Administration (FDA) on 21 December 2010 due to its association with an increased risk of myocardial ischaemia (angina, infraction). It was also withdrawn from the European market following recommendation by the European Medicines Agency (EMA, Sept, 2010) because of its cardiovascular-related side effects (http://www.indianexpress.com/news/after-ban-in-india-docs-warn-diabetics-of-rosiglitazone-use/694755/0). In Australia, rosiglitazone (Avandia, GSK) is marketed with a boxed warning that states: the use of Avandia is not recommended in patients with known ischaemic heart disease, particularly in those taking nitrates (http://www.diabetesaustralia.com.au/Media-Centre/Media-Releases/Avandia---Latest-News/). Pioglitazone hydrochloride (Actos) is an oral antidiabetic agent that acts primarily by decreasing insulin resistance but currently is not used clinically due to it having similar cardiovascular side effects to rosiglitazone; it also causes weight gain, anaemia and liver damage. Thiazolidinediones are potent ligands of PPARg2 and produce efficient insulin sensitization in type-2 diabetes patients.[63,64] *In vivo*, circulating levels of free fatty acids are reduced by thiazolidinediones. The thiazolidinediones also reduce insulin resistance in individuals with obesity.[8,59,60] TNF-α, which is produced by adipose tissue, influences the development of insulin resistance and promotes various disorders. The glycerol release and FFA release is increased by incubation of TNF-α with 3T3-L1 adipocytes. The incubation period is 24 h during TNF-α adipocyte lipolysis. Thiazolidinediones such as BRL 49653 reduced approximately 50% of TNF-α-induced glycerol and FFA released in adipocytes. BRL 49653 partially reduced the TNF-α mediated protein. There are two proteins, HSL and perilipins, involved in adipocyte lipolysis. Thiazolidinediones or glitazones are new class of drug for the treatment of type-2 diabetes. Some experimental data demonstrate the ability of thiazolidinediones to reduce FFA and increase insulin sensitivity.[65,66] Thiazolidinediones act as an antagonists for the effects of TNF-α. Thiazolidinediones binds with PPARg2 in adipocytes and enhances adipogenesis and fatty-acid uptake. PPARg2 is predominantly present in the liver and also mediates the triglyceride-lowering action of fibrates. It also improves sensitivity to insulin by reducing circulating fatty-acid concentration and lipid availability in liver and muscles.[67] Thiazolidinediones are highly agonistic to PPARg2 factor, which binds to the 9-*cis* retinoic acid receptor (RXR) to form a heterodimer. This heterodimer regulates gene transcription and translation of a variety of proteins that are involved in cellular differentiation, glucose and lipid metabolism.[32]

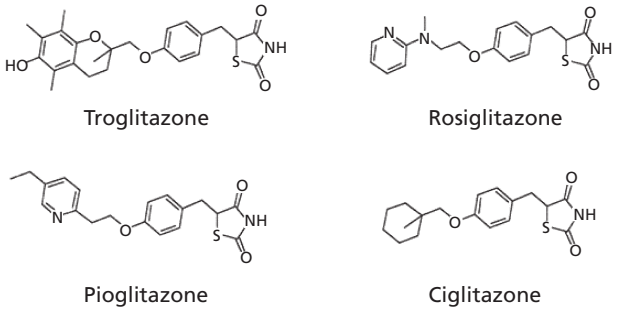

Figure 3 Chemical structures of drugs of the thiazolidinedione family.[61]

Thiazolidinediones as agonists of PPARg2 and insulin sensitivity

Thiazolidinediones improve insulin sensitivity and have emerged as an effective treatment for type-2 diabetes and other insulin-resistant states. They are potent ligands of PPARg2 and show efficient insulin sensitization in type-2 diabetic patients. Type-2 diabetes mellitus results from impairment in insulin secretion and resistance to insulin action. The impairment of insulin includes a subtype of maturity-onset diabetes of the young (MODY), maternally inherited diabetes with deafness (MIDD) caused by mitochondrial mutation

and insulin gene mutations. Major transcription protein associated with MODY include hepatocyte nuclear factor-α (HNF-α), insulin promoter factor-1 (IPF-1) and HNF-1β. They influence the expression of the other genes through regulation of mRNA synthesis. Type-2 diabetes mellitus has three monogenic forms, characterized by severe insulin resistance, which are the consequence of mutations in the *PPARg2* and insulin receptor genes.[68] The insulin-sensitizing effects of thiazolidinediones are mediated through PPARg2 transcription factor. Thiazolidinediones induce insulin sensitivity by controlling adipokines. Thiazolidinediones stimulate the PPARg2 by which thiazolidinediones down-regulate TNF-α, leptin, interlenkin-6 and plasminogen.[8,13] Upon stimulation, PPARg2 binds to responsive elements located in the promoter regions of many genes and modulates their transcriptive activity.[35,66,69] Thiazolidinediones bind with PPARg2 in adipocytes and enhance adipogenesis and fatty acid uptake (in peripheral fat). Currently, two thiazolidinedione drugs, rosiglitazone and pioglitazone, are prescribed clinically as antidiabetics. Evidence that PPARg2 is the insulin-sensitizing target of thiazolidinediones action includes a strong correlation between receptor binding and agonism and the biological potency of the glucose-lowering effect *in vivo*. Thiazolidinediones improve sensitivity of insulin through PPARg2 by reducing circulating fatty acid concentration and lipid availability in liver and muscles.[36,64,70] The thiazolidinedione derivative increased the expression of *adiponectin* mRNA in adipose cells and also increased plasma adiponectin concentration. Thiazolidinediones are synthetic ligands for PPARg2, which is a key factor that induces adipocyte differentiation by activating the expression of adipocyte-specific genes. Therefore, they are also kown as insulin sensitizers *in vivo*. The PPARg2 ligand promotes adipogenesis in cell culture and is also required for adipose development.[7,71,72] Thiazolidinediones directly minimize the systemic insulin resistance of peripheral tissues. There is a strong mutual relationship between receptor binding and agonism, which is evidence of the insulin-sensitizing target in PPARg2 of thiazolidindiones. This evidently increases the biological potency of the glucose-lowering effect of thiazolidindiones *in vivo* as well as their antidiabetic activity. The role of PPARg2 in glucose homoeostasis is due to mutation of *PPARg2* transcription factor. This mutation prevents phosphorylation on serine 112, which protects against obesity associated with insulin resistance. In the absence of PPARg2 transcription factor in fat, the muscle or liver development of insulin resistance is promoted.[5,64,70] Despite there being wide metabolic deposition of fat in different organs, adipose tissue is the basic target site for the glucose-lowering action of thiazolidindiones. In addition to this, thiazolidindiones are consistent in their glucose-reducing capacity in *PPARg2* specific knockout model of liver and muscle. Although thiazolidindiones directly modulate adipocyte glucose uptake, glucose disposal into adipose tissue contributes only modestly to the hypoglycaemic effect of insulin. The main insulin-sensitive organs are adipose tissue, muscles and liver, so these are the most targeted sites of thiazolidindiones. Insulin-sensitizing factor is influenced by thiazolidindiones, which reduce adipocyte expression of several insulin resistance-promoting polypeptides. Thiazolidindiones stimulate the PPARg2 by which it down-regulates TNF-α, leptin, interlenkin-6 and plasminogen.[8,13] Due to stimulation of PPARg2, it binds to responsive elements located in the promoter regions of many genes and modulates their transcriptive activity.[8,35,66,69] These factors lower the fatty acid level in serum by promoting flux into adipose tissue. All of these factors promote insulin sensitivity. The thiazolidindione's ligand with nuclear receptor, which is ligand-activated transcription factors, plays an integral part in the regulation of the expression of a variety of genes involved in carbohydrates and lipid metabolism. Thiazolidindiones are responsible for transcription of the *PPARg2* gene. Due to this activity, insulin sensitivity in type-2 diabetes mellitus is promoted (e.g. Figure 4).[73,74] The PPARg2 receptor is present to a lesser extent in muscle tissues. In the adipocyte, differentiation is enhanced but lipolysis is reduced and the level of circulating adipocytokines is altered.[73]

Control of Adipogenesis Signalling Pathway

Adipose cells send molecular signals to other tissues responsible for energy metabolism and adipogenesis. PPARg2 activation controls genes that regulate systemic insulin sensitivity. Two interesting candidate genes in this regard are *TNF-α* and *leptin*. The over-expression of *TNF-α* is present in obesity and insulin resistance. *PPARg2* activation by a PPARg2 ligand (thiazolidinedione) controls over-expression of these gene candidates. Thiazolidindiones block the ability of *TNF-α* to interfere with the most proximal events of insulin signalling. The important components of the *PPARg2* signalling system are endogenous ligands and enzymes that produce them, receptor levels and modifications, coactivators and corepressors and downstream transcriptional targets. A genetic defect in any of these aspects of this receptor system would result in reduced insulin action. The transcriptional activity of *PPARg* stimulated by thiazolidindiones can be sharply reduced due to phosphorylation, which is caused by the enzyme-activated protein kinase. These phosphorylated receptors show decreased affinity for ligand. Phosphorylation could alter interaction with important protein factors of *PPARg* such as coactivators and corepressors. Thus, nuclear receptors function as ligand-gated platforms for the assembly of these cofactors into large protein complexes on specific DNA sequences (50–52). Coactivator proteins (such as CBP/p300, SPC1 and pCAF) have histone acetyltransferase activity that functions to 'open' the configuration of chromatin and

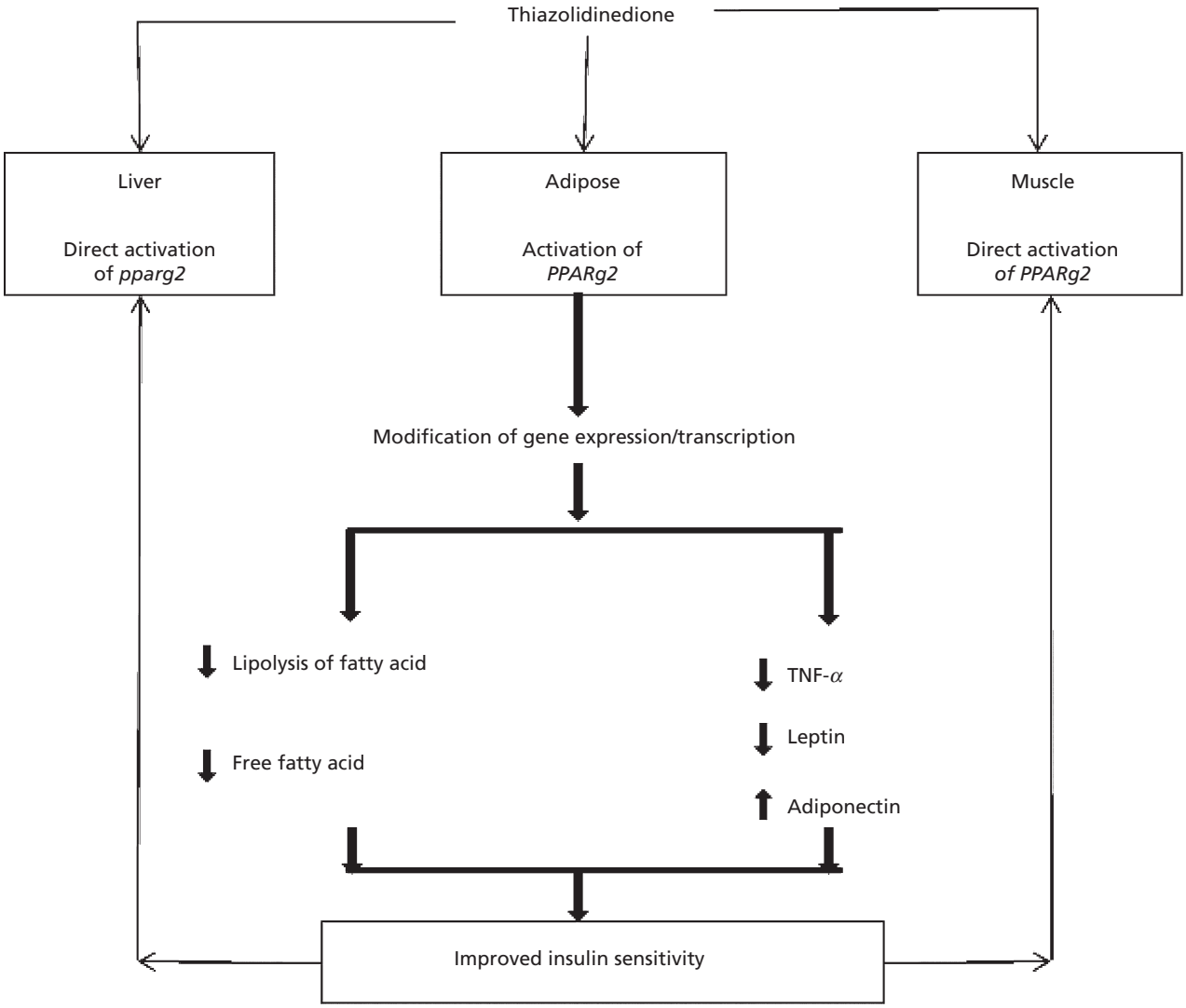

Figure 4 Mechanism of thiazolidinediones' action.[73] Thiazolidinediones are selective agonists for the peroxisome proliferator-activated receptor gamma (*PPARg*), which is most highly expressed in adipocytes and to a lesser extent in muscles and liver. In adipose tissue thiazolidinedione modify the gene transcription of the *PPARg2* gene by which it induces insulin sensitization. *PPARg2*, peroxisome proliferator-activated receptor gamma isoform-2; TNF-α tumour necrosis factor.

allows more efficient transcription.[75] There are two mechanisms: one is hypertrophy of individual adipocytes; the other is hyperplasia, due to the proliferation and differentiation of pre-adipocyte precursors, which are responsible for adipogenesis. The expression of thyrotropin receptor (TSHR) increases during adipogenesis, which is the main regulator of thyroid function and growth. TSHR activation induces pre-adipocyte differentiation. Retroviral vectors introduce constitutively active TSHR (TSHR*) in 3T3L1 pre-adipocytes. TSHR leads to phosphorylation of CREBP via cAMP/protein kinase A (PKA) signal. The activation of TSHR liberates two functional moieties of the Gs protein, α (which acts via PKA) and $\beta\gamma$ (signals via P13k). The former is a constitutively active Gsα (gsp*) that yields only the α subunit. Activating mutant human TSHR, L629F (TSHR*) and rat Gsα, Q227L (gsp*), are introduced using retroviral vectors in the laboratory. The Gsα subunit uses a gain-of-function mutation in a second component of the PKA pathway to achieve activation of CREBP which necessary for adipogenesis in 3T3L1 pre-adipocytes. Total FOXO1 (Forkhead box O1) protein expression is increased in type-2 diabetes mellitus. FOXO proteins are a subgroup of the Forkhead family of transcription factors. The members of class 'O', such as FOXO1, share characteristics of being regulated by the insulin/Pl3k/Akt

signalling pathway. FOXO1 phosphorylation (which is required to inactivate this repressor of adipogenesis) is lowest in gsp* despite the activation of AKT by phosphorylation. The G$\beta\gamma$ enhances down-regulation of pre-adipocyte factor-1 (PREF1-adipogenesis inhibitor) allowing retention of adipogenic potential in the TSHR population. Gsα signalling impedes FOXO1 phosphorylation and thus inhibits *PPARg* transcription and alternative promoter usage required to generate PPARg2, which is a fat-specific transcription factor necessary for adipogenesis. A protein, consisting of seven WD-repeats, forming a putative beta-propeller and an FYVE domain, propeller-FYVE (Professor), is highly expressed in 3T3L1 cells. It is a mediator that facilitates FOXO1 phosphorylation by phospho-AKT. Professor is a positive regulator of adipogenesis. G$\beta\gamma$promote adipogenesis by activation of PPARg2.[76]

Discussion

The goal of this review was to define the function of PPARg2 transcription factor, which interacts with thiazolidinediones to promote insulin sensitivity.[2–4] Lipid homoeostasis is an important determinant of adiposity and metabolic health.[77] The development of insulin resistance is dependent upon excess adiposity. It also acts as a key factor for broader a metabolic syndrome, which includes insulin resistance, abdominal obesity, elevated fatty acids, LDL, HDL cholesterol, cardiovascular disease and prothrombotic states.[78] Insulin resistance is associated with obesity, diabetes, hypertension, dyslipidaemia and atherosclerosis.[8] The release of FFAs from the adipose tissue can be done by lipolysis.[2] In state of insulin resistance, IRS phosphorylation, PI 3-kinase activity and GLUT-4α activity are impaired.[12] Elevated FFA results in over-expression of TNF-α, and several other proteins are expressed in fat tissue. These factors are responsible for the genesis of obesity-related insulin resistance.[8] TNF-α is mainly responsible for the development of insulin resistance.[8,14] Two distinct proteins, g1 and g2, arise by differential transcription start sites (TSSs) and alternative splicing.[31] The *Pro12Ala* variant (*rs1801282*) is the most common form of the *PPARg2* gene, which influences insulin sensitivity in type-2 diabetes. The *pro12Ala* substitution of *PPARg2* is associated with insulin sensitivity, low body mass index (BMI), high-density lipoprotein cholesterol levels and low total triglycerides.[79–81] It is also associated with low fasting insulin levels. It was found that sensitivity of both the glucose disposal and insulin sensitivity of lipolysis were greater in subjects containing Ala allele independent of the differences in age, sex, adiposity or fat distribution (waist-to-hip ratio). On a theoretical basis, the alteration of transcriptional activity of the Ala variant of the *PPARg2* gene depends upon thiazolidinediones' agonist action by which they enhance the insulin signalling in muscle as well as in adipose tissue. It is clear that PPARg2 induces the genetic program that is used as a source of fatty acids for triglyceride formation in adipocytes. PPARg2 also causes a genetic transformation in glycerol homoeostasis.[17] This results from the suppression of lipolysis by thiazolidinediones, causing a reduction of FFAs. Insulin-sensitizing factor is derived from thiazolidinediones, which reduce adipocyte expression of several insulin resistance-promoting polypeptides. Thiazolidinediones stimulate the PPARg2 by which thiazolidinediones down-regulate TNF-α, leptin, interlenkin-6 and plasminogen.[8,13] When stimulated, PPARg2 binds to responsive elements located in the promoter regions of many genes and modulates their transcriptive activity.[8,35,66] PPARg2 transcription factor is also present in liver and muscles, because of this; thiazolidinediones also improve insulin sensitization in liver and fat tissues by reducing lipid availability and circulating fatty acids.[67] Thiazolidinediones bind with PPARg2 in adipocytes and enhance adipogenesis and fatty acid uptake. Evidence that PPARg2 is the insulin-sensitizing target of thiazolidinediones' action includes a strong correlation between receptor binding and agonism and the maintenance of the glucose lowering effect as well as antidiabetic activity. So the role of PPARg2 transcription factor is very important in type-2 diabetes. This transcription helps thiazolidinediones in the induction of insulin sensitization.[5,82,83]

Conclusions

It can be summarized that PPARg2 acts as a regulator for insulin sensitization in type2 diabetes. The variant *Pro12Ala* (*rs1801282*) of the *PPARg2* gene promotes the activity of thiazolidinediones to minimize insulin resistance. Thiazolidinediones are important agonists for PPARg2 transcription factor, which helps the *PPARg2* in transcription of the gene. Because of this, there is a strong correlation between receptor binding and agonism and control of the glucose-lowering effect as well as antidiabetic activity. Transcriptional activity of the *Pro12Ala* variant improves the activity of insulin. Thus the specific modulation of these characteristics of *PPARg2* permits the modulation of tissue and target gene specificity. *PPARg2* improves the insulin sensitivity by coordinating with thiazolidinediones, which cause activation of PPARg2 receptor and decrease insulin resistance by maintains levels of TNF-α and fatty acids in adipose tissue. Thiazolidinediones enhance the expression and secretion of adiponectin through the activation of PPARg2 and antagonize the suppressive effect of TNF-α on the production of adiponectin. Hence activation of PPARg2 enhances the therapeutic efficacy of thiazolidinediones, especially in metabolic diseases. PPARg2 was recently found to play an important role in inflammation, bone morphogenesis, insulin sensitivity, endothelial cell function, cancer, longevity and atherosclerosis.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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