Insulin resistance precedes the development of type 2 diabetes mellitus and is also a common denominator in the so-called metabolic syndrome. Although the cause of insulin resistance has not been fully elucidated, it seems clear that lifestyle changes, including little physical exercise and constant access to food, particularly in developed and economically emergent countries, as well as genetic factors, appear to have triggered the escalating incidence of diseases related to insulin resistance, including type 2 diabetes and metabolic syndrome. Obesity is considered as a risk factor for developing insulin resistance. Increased adipose tissue has been related to an increased production of pro-inflammatory cytokines which, together with fatty acids, appear to be responsible for the development of insulin resistance. Thus, a greater or lesser expansibility or ability of adipose tissue to store lipids also appears to play a significant role in the development of insulin resistance because overcoming of this capacity, which is variable in each case, would result in leaking of lipids to other tissues where they could interfere with insulin signaling. This article reviews various molecular mechanisms related to the development of insulin resistance and its relationship to expansibility of adipose tissue and obesity.

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tejido adiposo se ha relacionado con el aumento de la producción de citoquinas proinflamatorias, que junto a los ácidos grasos, parecen ser los responsables del desarrollo de la resistencia a insulina. Así, la mayor o menor expansibilidad o capacidad del tejido adiposo para almacenar lípidos también parece jugar un papel importante en el desarrollo de la resistencia a insulina ya que la superación de esta capacidad, variable en cada caso, sería la causa del escape de lípidos a otros tejidos donde podrían interferir con la señal de insulina. En este artículo se repasan diversos mecanismos moleculares relacionados con el desarrollo de la resistencia a insulina y su relación con la expansibilidad del tejido adiposo y la obesidad.

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Introduction

Insulin resistance is characterized by a decreased capacity of insulin to carry out its normal physiological functions. Insulin resistance usually precedes overt pathological conditions such as type 2 diabetes mellitus or metabolic syndrome, and is associated to circumstances such as excess weight or obesity. Mention could also be made of other circumstances, such as age, pregnancy, and polycystic ovary syndrome, where insulin resistance also plays a significant role. Insulin resistance initially induces compensatory mechanisms, so that insulin hypersecretion maintains blood glucose under control for some time. This stage, which may be called prediabetic, is clinically difficult to detect just because normal blood glucose values are maintained. However, the situation gradually impairs due to the occurrence of the so-called pancreatic failure, in which beta cells not only are unable to maintain insulin hypersecretion but also start to deteriorate, and insulin secretion is decreased. This is the point at which most cases of type 2 diabetes mellitus and metabolic syndrome start to be diagnosed. An alternative for earlier detection of resistance would be testing of blood insulin levels, either in fasting conditions to calculate the resistance index as measured by the homeostasis model assessment (HOMA) or in glucose tolerance curves to detect hyperinsulinemia. Progression of insulin resistance not only leads to type 2 diabetes. If adequate measures are not taken, patients eventually become insulin-dependent. While the cause of insulin resistance has not been clearly elucidated yet, it is thought that a polygenic genetic component exists on which the environment would act. In this regard, lifestyle changes including little physical exercise and constant access to food in developed and economically emergent countries appear to be responsible for the escalating incidence of diseases related to insulin resistance, such as type 2 diabetes, in recent years. More than 90% of diabetic patients are considered to have type 2 diabetes. According to the World Health Organization, the number of people affected by this disease worldwide was expected to increase from 135 million in 1999 to 299 million in 2025. Such predictions appear to be an underestimation, and according to the International Diabetes Federation, incidence would increase from 246 million in 2007 to 380 million in 2025. If we add to this the progressive aging of the population in developed countries, which increases the prevalence of type 2 diabetes and metabolic syndrome, it becomes obvious that we face a significant healthcare problem that requires maximum attention. It is predicted that virtually 30% of the population will experience insulin resistance and its complications during their lifetime.

On the other hand, as regards the genetic bases related to development of diabetes and obesity, it should be noted that in the past three years, genome-wide association studies (GWAS) have had an unprecedented success, identifying loci involved in common diseases such as obesity and diabetes. For example, more than 35 susceptible loci have been identified to date for type 2 diabetes and 32 for obesity. In 2006, transcription factor 7-like 2 (TCF7L2) was implicated in type 2 diabetes. New loci have subsequently been identified, as well as putative candidate genes associated to such loci whose implication in insulin secretion, rather than in insulin action, has been confirmed. GWAS for obesity has provided similar results: 18 loci identified for body mass index and 13 loci identified for body fat in 2010. Most of these loci contain genes pointing to the role played in the disease by neurons and brain, particularly hypothalamus, while a lower number would have an influence upon adipocyte biology.

These genetic and epidemiological data speed up the study of mechanisms regulating insulin sensitivity. Insulin not only regulates glucose homeostasis but also has a significant role in lipid a protein metabolism, which may in turn be impaired in insulin resistance states. Due to the complexity of the system, studies have not only aimed at in vitro characterization of insulin signaling pathways and development of in vivo models to be able to establish any physiological correlation but also at examining other extrinsic factors affecting the various molecules in the insulin signaling cascade which may be involved in development of insulin resistance.

Factors involved in development of insulin resistance

Insulin resistance may occur due to some abnormality in the insulin signaling cascade. In an initial attempt to ascertain the modulators of insulin sensitivity, the first approaches consisted of use of animal models with deletions in the genes involved in the insulin signaling cascade. Normal insulin signaling occurs through activation of an insulin-specific receptor belonging to the tyrosine kinase receptor subfamily. Knockout mice homozygous for the
insulin receptor die within one week of life due to marked ketoacidosis. However, knockout mice heterozygous for the insulin receptor are viable, and approximately 10% eventually experience diabetes. Unlike most tyrosine kinase receptors, activated insulin receptor directly phosphorylates insulin receptor substrate (IRS)-1-4 into multiple tyrosine residues. Four members of the IRS have been reported to be involved in insulin signaling, of which IRS-1 and 2 are the most important in glucose transport. IRS1 deletion (IRS1KO) results in small mice showing insulin resistance in muscle, which is compensated for by pancreatic beta cell hyperplasia. Knockout mice for IRS2 have a phenotype of severe insulin resistance in the liver and diabetes at an early age. Unlike IRS1KO mice, however, this model shows no compensatory beta cell hyperplasia because the pancreatic duodenal homeobox transcription factor (Pdx)-1, required for insulin gene transcription, is regulated by IRS2.

The next insulin signaling level involves phosphoinositide 3-kinase (PI3-kinase). PI3-kinase is a key element in metabolic response to insulin that regulates glucose transport, antilipolytic effect, fatty acid synthesis, and glycogen synthesis. There are various isoforms of regulatory subunits (p85α, p55γ, p50α, p85β, p55γ) and catalytic subunits (p110α, β). As examples of mouse models at this level, complete deletion of regulatory subunits p85 causes death after birth. However, heterozygous mice with a decrease in regulatory subunits p85α, β55, and 50α have an increased insulin sensitivity leading to hypoglycemia.

Protein kinase B (PKB/Akt) belongs to a lower level in regulation of insulin signaling. There are three different isoforms of this enzyme, of which only Akt2 appears to mediate insulin sensitivity in skeletal muscle and liver. Akt2 deletion causes insulin resistance in liver and skeletal muscle and induces diabetes. By contrast, deletion of Akt1/PKBα does not cause insulin resistance or glucose intolerance, although the deficiency causes growth retardation in these animals. These animals therefore suggest that Akt2 is the essential isoform for maintenance of glucose homeostasis.

The final effect of insulin is to facilitate glucose transport through the glucose transporter 4 (Glut 4). Glut 4 heterozygous knockout mice show glucose intolerance, insulin resistance, and hypertension, but do not suffer diabetes. In fact, homozygous mice with Glut 4 deletion do not experience diabetes at an early age. This suggests that other compensatory mechanisms occur at early ages, since only 50–60% of mice reaching adult life develop diabetes. In addition to Glut transporters, insulin-mediated glucose transport requires other complex proteins that facilitate passage of Gluts from intracellular vesicles to plasma membrane. Changes in these molecules may trigger insulin resistance, as seen in knockout mice for the target-SNARE (soluble NSF attachment protein) receptor (t-SNARE) syntaxin 4 protein, which show glucose intolerance and a decreased glucose uptake by skeletal muscle. At any rate, although these animal models have helped in the study of factors involved in molecular pathogenesis of insulin resistance, very few patients with monogenic syndromes show extreme phenotypes of insulin resistance. Considering the multiple effects of insulin resistance in the different organs and the multiple environmental factors implied development of resistance, the final objective is to act on multiple targets, rather than only one of them. This strategy will allow for success when designing potential drugs for prevention and treatment of insulin resistance.

**Obesity and inflammation in development of insulin resistance**

Insulin resistance may also occur due to other factors which are able to interfere with or modify in any form some of the molecules involved in this pathway. It has been known for some time that there are epidemiological data associating insulin resistance to inflammatory markers and that high salicylate doses also contribute to decrease blood glucose in diabetic patients. However, reports of the relationship between proinflammatory cytokines and insulin resistance or that the signaling pathway mediated by the B kinase inhibitor (IKKβ)/nuclear factor κB (NF-κB) (IKKβ/NF-κB) was one of the salicylate targets are relatively recent. It has been shown in recent years that adipose tissue hypertrophy and hyperplasia may cause hypoxia, and activation of different cell responses including oxidative stress, endoplasmic reticulum stress, and inflammation. Although these responses have often been independently studied, there are increasing data that relate them to each other. As regards inflammation, it has been reported that expansion of adipose tissue not only increases macrophage infiltration in adipose tissue but also causes a change in polarization of macrophages, which would convert from type M2 macrophages, with an anti-inflammatory secretory profile, into M1 macrophages, having a proinflammatory secretory profile. This latter type would be responsible for expression of most proinflammatory cytokines produced in adipose tissue and molecules involved in recruitment of additional macrophages into tissue, thus creating a vicious cycle that would amplify activation of inflammatory pathways. Although the mechanisms involved in macrophage recruitment and change in polarization of macrophages have not been fully elucidated, it is known that the monocyte chemoattractant protein 1 (MCP1/CCL2) produced by macrophages and adipose tissue plays a significant role in the process. This protein acts through its chemokine (C–C motif) receptor 2 (CCR2), and they both appear to be increased in adipose tissue of obese animals. On the other hand, the molecular mechanisms that would explain the inhibitory action of proinflammatory cytokines on insulin action appear to be located interfering with the insulin signaling pathway at steps subsequent to insulin binding to the receptor. Proinflammatory cytokines such as tumor necrosis factor-alpha (TNFα) stimulate phosphorylation of IRS1 to serine, cause defects in the tyrosine kinase activity of IR, and decrease activity of IRS1 and P13 kinase, thus inhibiting the insulin signaling pathway.

Among the serine/threonine kinases activated through the inflammatory pathways, mention should be made of Jun N-terminal kinase (JNK), IKKβ/NF-κB, and protein kinase C (PKC). Moreover, some of these kinases, such as JNK, may also be activated in response to other alarm signals such as oxidative or endoplasmic reticulum stress. On the other hand, free fatty acids could also contribute to inhibition of insulin signal by activating toll-like receptors (TLRs), which would...
also activate signaling pathways such as JNK or IKKβ/NF-κB. These would in turn interfere with insulin signal as discussed above. TLRs play a crucial role in the innate immune system by detecting the presence of pathogens. This is a wide receptor family, of which at least 12 members showing different pathogen recognition preferences are currently known.40 One of the best characterized of these members is TLR4, expressed in various cell types, but mainly in cells of the innate immune system such as macrophages and dendritic cells. This receptor recognizes lipopolysaccharides (LPS) in the cell wall of Gram-negative bacteria and activates not only pathways such as those mediated by IKKβ/NF-κB or JNK but also pathways such as MAP kinases leading to activation of transcription factors such as activating protein 1 (AP-1), mediating expression of proinflammatory cytokines.40 TLR2 is another type of receptor implicated in this type of mechanism.41 Data available show that some fatty acids, particularly medium-chain saturated acids such as palmitate or stearate, may act as TLR agonists, which would explain the harmful effect of fatty acids on insulin action.33,42-44 Although an increased TLR expression in muscle has also been reported in insulin-resistant subjects,45 some authors have questioned the direct effect of free fatty acids, attributing their inhibitory effects on insulin signal to contaminants such as LPS and lipopeptides present in the albumin used to perform in vitro experiments. Alternatively, it has been suggested that fatty acids may play an indirect role, acting as precursors of ceramides and sphingolipids, whose inhibitory effects upon insulin action have also been reported.46 Thus, either directly or indirectly, increased levels of free fatty acids resulting from expansion of adipose tissue, combined with an increased production of proinflammatory cytokines, would be responsible for development of insulin resistance not only in adipose tissue but also in other peripheral tissues where these molecules may also exert their inhibitory effect upon insulin action.

The potential role that both fatty acids and proinflammatory cytokines may have in the development of central resistance to insulin and leptin may also be discussed. These effects could have a greater impact because these are both lipostatic signals, and their impairment could therefore be on the basis of the genesis of obesity. There is currently no doubt about the role of leptin and insulin as lipostatic signals targeting areas of the central nervous system such as hypothalamus, regulating calorie homeostasis through energy intake and expenditure.47 Impairment of, or central resistance to, these signals could therefore be in the basis of obesity triggers, which would in turn feed back this vicious cycle, exacerbating central and peripheral resistances as obesity progressed. Moreover, the fact that these signals regulate different aspects of peripheral metabolism such as hepatic glyconeogenesis48 and peripheral insulin sensitivity49 supports their importance for peripheral insulin sensitivity and their impact on control of overall calorie homeostasis. Central resistance to insulin and leptin has been reported in various experimental models associated to situations of overall insulin resistance such as aging50 or obesity induced by fat-rich diets.51,52 In the latter, insulin resistance occurs associated to an increased inflammation in hypothalamus.53 The fact that inhibition of the IKKβ/NF-κB pathway54 or signaling mediated by TLR4 receptors45,55 in hypothalamus reverses the inhibitory effect of fatty acids upon insulin and leptin resistance agrees with the concept of the role of fatty acids and inflammation in the mechanism of central resistance to insulin.

Implication of endoplasmic reticulum stress and oxidative stress in insulin resistance

Endoplasmic reticulum (ER) stress56,57 and oxidative stress (OS)58-60 are conditions which have also been related to insulin resistance and may be implicated in some of the changes associated to diabetes and other diseases. ER is an organelle involved in protein synthesis and traffic. For this, proteins should properly be folded with the help of chaperones. When ER is not able to adequately fold and export proteins for various reasons, associated to an effort or cell stress condition, the so-called ER stress occurs. This triggers the so-called unfolded protein response (UPR), including various responses at transcriptional levels such as increased chaperone synthesis, protein synthesis inhibition, and activation of protein degradation via proteasome, all of them aimed at restoring balance. Signals able to activate reticulum stress (RS) include calcium availability, presence of pathogens, or changes in nutrient availability. That is, RS could be considered as a sensor that detects changes in cell homeostasis. In this regard, RS has been associated to obesity and insulin resistance57 in conditions which increased protein and lipid synthesis, as well as accumulation of lipids and the resultant changes in cell structure, would activate this cellular response. This occurs in various tissues, including adipose tissue, liver, and pancreas. In the pancreas, insulin hypersecretion to maintain normal glycemia in resistance situations could be one of the causes contributing to dysfunction of pancreatic β cells. RS, combined with the abovementioned responses, causes an increase in JNK and IKKβ/NF-κB activities, which may promote phosphorylation of IRS to serine, inhibiting insulin signal. In addition, this also provides a link between RS and inflammation signaling pathways.

OE is apparent as an increase in reactive oxygen species (ROS) resulting from an imbalance between systems producing them, such as mitochondrial activity or activities such as NADPH oxidase (NOX), and systems removing them such as superoxide dismutase or catalase. In addition to altering a great variety of cell structures due to their chemical reactivity, ROS also induce inflammatory responses and have been related to insulin resistance and diabetes. Potential causes of oxidative stress include hyperglycemia or high fatty acid levels, conditions which are associated to high-calorie diets.62 In addition, oxidative stress activates signaling pathways such as JNK and IKKβ/NF-κB, p38 MAPK, or PKC δ which, as discussed above, may negatively modulate the insulin signaling pathway.62-64

An additional factor related to oxidative stress and to development of insulin resistance is mitochondrial dysfunction. A decrease in the oxidative capacity of mitochondrion and particularly of fatty acids may promote accumulation of acyl CoAs and diacylglycerol, which may in turn activate serine/threonine kinases which, by phosphorylating receptor or insulin receptor substrate, would interfere with this signal, causing insulin resistance.58 On the other hand, mitochondrial function also plays a very significant
role in the mechanism of insulin secretion by pancreatic β cells in response to increased glucose levels. In addition to some genetic defects related to mitochondrial dysfunction which have been associated to impaired insulin signaling such as MELAS (myopathy, encephalopathy, lactic acidosis, and stroke), due to a mutation in mitochondrial DNA, mitochondrial dysfunction has also been associated to insulin resistance.⁶⁄⁻⁶⁷ In this regard, it should be noted that changes in the number, size, and oxidative capacity of muscle mitochondria have been reported in patients with diabetes or insulin resistance.⁶⁵ This was also demonstrated during aging when elderly subjects with insulin resistance were shown to have a decrease in both the number and oxidative capacity of muscle mitochondria as compared to young subjects without insulin resistance.⁶⁶ Mitochondrial dysfunction has been related to a decreased expression of genes involved in oxidative phosphorylation regulated through co-activator 1α of peroxisome proliferator-activated receptor-gamma (PPARγ) (PGC-1) in muscle from diabetic patients,⁵⁹⁄⁻⁶⁸³ demonstrating the role of PGC1α in both mitochondrial biogenesis and function. In fact, some mutations in PGC1α associated to type 2 diabetes have been reported,⁶⁹⁻⁷⁰ PGC1β, the PGC1α homolog, is mainly expressed in muscle and heart, tissues with a high content in mitochondria.⁷¹⁻⁷² The role of PGC1β in pathogenesis of insulin resistance induced by fructose has recently been shown, and it has therefore been suggested that inhibition of the PGC1β isofrom could be a target for treatment of hypertriglyceridemia and insulin resistance associated to an increase in de novo lipogenesis.⁷³

Based on the foregoing, a potential approach for preventing development of insulin resistance and diabetes induced by the toxic effects of overnutrition would be to promote oxidative capacity of muscle by increasing mitochondrial count through an increased PGC1α expression. In fact, PGC1α overexpression in muscle promotes development of pro-oxidative red fibers associated to an increased mitochondrial count. On the other hand, the feasibility of this concept of increasing fatty acid oxidation as a strategy for preventing insulin resistance may be demonstrated in transgenic animal models overexpressing both mitochondrial uncoupling protein (UCP) 1⁷⁴ and UCP3.⁷⁵ Mitochondria of these animals are uncoupled, and therefore oxidize more fatty acids. These animals are thin and more sensitive to insulin, which suggests that this type of strategy designed to avoid excess energy may have beneficial and positive effects on insulin sensitivity. A recent study using animals overexpressing UCP3 in muscle and with similar weights showed an improvement in insulin sensitivity. This shows that an increased metabolic rate and fatty acid oxidation affects insulin sensitivity regardless of body weight.

**Effect of lipotoxicity: the paradox of insulin resistance in lipodystrophy and obesity**

As mentioned above, free fatty acids may directly or indirectly interfere with insulin signal, which has led to the concept of lipotoxicity as a result of ectopic accumulation of lipids and their harmful effects on different tissues other than adipose tissue, the one in which lipids usually accumulate.⁷⁶⁻⁷⁸ This concept is usually handled in the setting of overweight and obesity.⁷⁹ It should be noted, however, that insulin resistance also occurs in non-obese subjects, and that there is a population of obese individuals who have no significant metabolic changes or signs of insulin resistance. This does not necessarily contradict the concept of lipotoxicity itself, but suggests the different capacity of individuals to maintain a non-ectopic lipid accumulation. That is, while a subject is able to maintain adipose tissue expansion with no ectopic accumulation of adipose tissue, he/she will be able to maintain metabolic normality without developing insulin resistance. This limit may change depending on the subject, and it is obvious that the greater the adiposity the higher the chance of reaching the limit, which explains the higher rate of insulin resistance and type 2 diabetes in obese subjects. It is less intuitive to explain how lipodystrophy, a phenotype that may be considered as the opposite to an obesity status, may also be related to insulin resistance. Lipodystrophy could illustrate the case of development of insulin resistance due to impossibility of adipose tissue expansion. In both cases, fat accumulates ectopically in liver, muscle, and pancreatic beta cells, where a lipotoxic effect leading to insulin resistance occurs.⁷⁹

In recent years, animal models have been described which are being crucial to be able to explain these two concepts of lipotoxicity and adipose tissue expansion. For example, many studies have shown that deletion of TNFα and/or its receptor prevents development of insulin resistance and is associated to a decrease in fatty acids and their lipotoxic effect, with an improvement in insulin signaling in skeletal muscle and adipose tissue.⁸⁰ Adiponectin or adipocyte complement-related protein of 30 kDa (ACRP30) is another hormone secreted by adipose tissue having positive effects on insulin signaling and which has been related to insulin sensitivity and adipose tissue expansion. Expansion of the ACRP30 gene is decreased in insulin resistance associated to obesity, and decreased expression returns to normal levels when insulin sensitivity is increased. It is therefore not strange that knockout mice for ACRP30 develop insulin resistance when fed a fat-rich diet.⁸¹ These animals have high fatty acid and TNFα levels in plasma and defects in PI3 kinase in the insulin signaling cascade. There is a very interesting mouse model to be able to explain the hypothesis of adipose tissue expansion and lipotoxicity, the mouse overexpressing adiponectin in adipose tissue (AdTg mouse). When these mice were crossed with leptin-deficient obese (ob/ob) mice, the resulting AdTg-ob/ob mice showed an increased body mass as compared to obese ob/ob mice but, despite this, better insulin sensitivity as compared to obese mice.

The study of mechanisms involved in regulation of adipose tissue expandability represents an interesting challenge and may also have a clinical impact. There are different possibilities for preventing lipotoxicity and, thus, different approaches for prevention of insulin resistance. The first and most commonly recommended by experts in obesity is a decreased dietary intake (fatty acids and carbohydrates). The second option, as previously discussed, is an increased mitochondrial oxidation of fatty acids so that they are not stored in the cell. Exercise is the most physiological way to oxidize fatty acids, and would therefore potentially prevent lipotoxicity. In addition, there are also drugs such as fibrates (used to decrease triglyceride levels) or metformin
that could be used in this strategy to prevent lipotoxicity and insulin resistance. Despite this, exercise and diet have also been more effective for the treatment of progression from fasting glucose intolerance to diabetes.\textsuperscript{64} Exercise, even for short time periods, also improves insulin sensitivity in patients with no significant weight loss.\textsuperscript{83,84} There is evidence that, in the lipotoxic phenomenon, the type of ectopic lipids is more important than the amount of lipids accumulated in the various tissues. In this regard, treatment of insulin resistance would be possible by directing ectopic accumulation of these lipids toward healthier forms (e.g. triglycerides) rather than more harmful forms such as ceramides, diacylglycerols, or lysophosphatidylcholines. This has been shown to have a beneficial effect in muscle by increasing expression of the enzyme diglyceride acyltransferase (DGAT)\textsubscript{1}, increasing triglyceride synthesis and decreasing insulin resistance induced by obesity.\textsuperscript{85} By contrast, reduction in DGAT\textsubscript{2} expression in the liver with the resultant decrease in lipid accumulation as triglycerides causes an increased toxicity by increasing hepatic fibrosis in a model of non-alcoholic steatohepatitis.\textsuperscript{86} Finally, the last strategy consists of increasing the storage capacity of adipose tissue by acquisition of new adipocytes. The aim is to increase adipocyte size, rather than adipocyte count. Insulin resistance of adipose tissue appears to be due to adipocyte size rather than to adipocyte count and, for example, distribution of the same amount of fat between higher numbers of cells causes a decrease in TNF\textalpha levels and insulin resistance. This was until recently one of the principles of treatment of type 2 diabetes with thiazolidinediones (TZDs) which, by acting upon the PPAR\gamma receptor, facilitate differentiation of new adipocytes.

**Role of PPAR\gamma in adipose tissue expansion and insulin sensitivity**

Peroxisome proliferator-activated receptors (PPARs) are transcription factors activated by lipid derivatives that regulate aspects of metabolism such as fatty acid oxidation, adipogenesis, and insulin sensitivity. The PPAR family consists of three members, PPAR\textalpha, \gamma, and \delta.\textsuperscript{87} PPAR\gamma is the receptor for the drugs improving insulin sensitivity known as TZDs. Paradoxically, in addition to improving insulin sensitivity, PPAR\gamma activation plays a role in adipocyte differentiation. In order to understand the molecular mechanisms leading to this paradox, various groups have generated different models of global knockout animals for PPAR\gamma. However, such efforts failed, as these animals were found to be non-viable due to inadequate placental vascularization. However, the study of heterozygous mice was able to find that when these animals were fed a fat-rich diet, their weight did not increase and, most surprisingly, they were more sensitive to insulin, apparently by a mechanism associated to increased leptin levels. Other animal models have provided new data showing the significance of this receptor in insulin sensitivity. Two of these, PPAR\gamma hypomorphic mice\textsuperscript{88} and mice with adipose-specific PPAR\gamma deletion,\textsuperscript{89} show congenital and progressive lipodystrophy. Fat accumulation is impaired in these mice, which accumulate fatty acids in tissues other than adipose tissue, and therefore develop insulin resistance which is associated to a lipotoxic effect. Although PPAR\gamma is mostly expressed in adipocytes, it is also found in macrophages.\textsuperscript{106,107,109} PPAR\gamma and PPAR\delta have been shown to be involved in various aspects of the macrophage activation program from a M1 state to a M2 state, characterized by being more dependent on fatty acids.\textsuperscript{90,91} Studies on macrophage-specific PPAR\gamma knockout mice, which show insulin resistance, demonstrated that macrophages could be very important target cells for the antidiabetic action of TZDs.\textsuperscript{92}

PPAR\gamma is expressed as three transcripts encoding two different proteins, PPAR\gamma\textsubscript{1} and PPAR\gamma\textsubscript{2}.\textsuperscript{93} PPAR\gamma\textsubscript{1} is expressed in different tissues including liver and muscle, while PPAR\gamma\textsubscript{2} is mainly expressed in white and brown adipose tissue under normal conditions, and more specifically in mature adipocytes. Various studies in adipocyte cell lines\textsuperscript{84} and an increased expression in non-diabetic obese subjects\textsuperscript{95} suggested that isoform \gamma\textsubscript{2} of PPAR\gamma was the most adipogenic isoform. Two different mouse models with the same deficiency in the specific isoform \gamma\textsubscript{2} of PPAR\gamma (PPAR\gamma\textsubscript{2} knockout mice) were generated.\textsuperscript{96,97} In white adipose tissue of PPAR\gamma\textsubscript{2} knockout mice, expression of genes related to adipogenesis was decreased, and although total lipid mass was maintained in one of the models, there was also a decrease in long-chain triglyceride levels. This effect triggers increases in other lipid species such as short-chain triglycerides, diacylglycerols, phospholipids, and ceramides which could account for development of insulin resistance. Although these data suggest that isoform PPAR\gamma\textsubscript{2} is required for maintenance of insulin sensitivity, these mice had no obvious metabolic phenotype until they were crossed with obese ob/ob mice to obtain double knockout (POKO).\textsuperscript{98} POKO mice have a 10–20% greater fat mass as compared to wild control mice, but less than half the fat as compared to obese ob/ob mice. Despite being thinner than obese mice, POKO mice are resistant to insulin from a very young age and develop diabetes, showing a failure in proliferation of pancreatic beta cells.\textsuperscript{99}

Expression of PPAR\gamma\textsubscript{2} has been shown to be greater in young as compared to older subjects.\textsuperscript{100} Decreased PPAR\gamma\textsubscript{2} expression in adipose tissue during aging could promote accumulation of lipotoxic species in tissues other than adipose tissue\textsuperscript{101,102} and lead to insulin resistance and mitochondrial dysfunction.

On the other hand, patients with mutations in the PPAR\gamma receptor have also been characterized.\textsuperscript{103} Individuals heterozygous for the dominant-negative mutation in PPAR\gamma (P467L) show a significant reduction in body mass and severe insulin resistance. The mouse model of the human P467L mutation (corresponding to the P465L mutation in mice) was also crossed with ob/ob mice.\textsuperscript{104} As compared to obese ob/ob mice, these mice have decreased body mass and insulin sensitivity. Both POKO mice and the P465L dominant-negative mutation model show an increase in body fat mass as compared to thin mice, but have less adipose tissue than obese ob/ob mice. These mouse models, having a decreased adipose tissue expandability and greater insulin resistance as compared to ob/ob mice, could be compared to patients who are moderately overweight and show a greater insulin resistance than more obese patients. Both models may explain the concept that a genetic limitation for adipose tissue expansion under conditions of positive energy balance may lead to insulin resistance and other metabolic complications.
Conclusions and future prospects

There is increasing evidence showing that various conditions such as obesity, inflammation, or oxidative or ER stress have a more significant role in pathogenesis of insulin resistance and type 2 diabetes. Such diversity suggests that maybe we should not speak of a single cause of insulin resistance, as it may be expected that additional causes and/or mechanisms related to insulin resistance are reported in the future. Understanding of the different factors triggering insulin resistance and their mechanisms and interrelations may lead us to be able not only to more precisely pinpoint the etiology in each patient, but also to administer more selective treatment. Although significant advances have been made in the knowledge of the action mechanisms of insulin and their changes, there is much more to be learned about them.

Conflicts of interest

The authors state that they have no conflicts of interest.

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