Insulin resistance is the most important pathophysiological feature in many pre-diabetic states, in which target cells fail to respond to normal levels of circulating insulin. In addition, insulin resistance is a key component together with obesity, hypertension, hyperlipaemia and hyperglycaemia of the metabolic syndrome or insulin resistance syndrome, with the outcome of cardiovascular disease. Type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus, NIDDM) is a complex metabolic disease with environmental and genetic components affecting over 5% of the population in Western societies. The pathogenesis of type 2 diabetes involves abnormalities in both peripheral insulin action and insulin secretion by pancreatic \( \beta \)-cells. In fact, some pro-inflammatory signals, such as tumour necrosis factor (TNF)-\( \alpha \), induce insulin resistance associated with chronic infections or obesity. Insulin resistance is usually compensated by
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hyperinsulinemia. Although moderate hyperinsulinemia might be tolerated in the short-term, chronic hyperinsulinemia exacerbates insulin resistance and contributes directly to β-cell failure and diabetes (Lee & White 2004).

**Genetic models for the study of insulin resistance and sensitivity**

Transgenic and knockout technology used to create animal models of type 2 diabetes have had a major impact on assessment of the function of insulin signalling molecules implicated in the regulation of glucose homeostasis in vivo (Kadowaki 2000, Nandi et al. 2003). Of particular impact on the understanding of type 2 diabetes has been the generation of knockout mice using the Cre-LoxP technology, to define the specific role of the insulin receptor (IR) in insulin target tissues. The creation of genetically manipulated mice models in a tissue-specific manner allowed us and others to elucidate the tissue specificity of insulin action, its contribution to overall insulin resistance and, finally, the global compensatory mechanisms induced in response to the genetic challenge (Kitamura et al. 2003). In addition, we have used those genetically manipulated mice as a source of established cell lines, in which the molecular mechanisms underlying the tissue-specific insulin resistance or sensitivity have been sorted out.

**Insulin resistance candidate genes**

The endocrine metabolic frame of insulin resistance is a characteristic from NIDDM, gestational diabetes, polycystic ovary-related diabetes, obesity-related diabetes and other diabetic syndromes (Roth 1994). The insulin hypersecretion and pancreatic β-cell hyperplasia could degenerate in a characteristic insulin insufficiency of type 1 diabetes. Type 1 diabetes affects 5% of the overall diabetic patients. The rest of the diabetic syndromes affect 95% of the entire diabetic population, and type 2 diabetes (NIDDM) is prevalent in a majority of these patients. Insulin-dependent diabetes mellitus is a predetermined autoimmune genetic disease. Although of unknown aetiology, type 2 diabetes is considered a predetermined genetic disease too. In both cases, it is thought that both diseases are of polygenic origin (Kahn 1995). During the last years, a new insight into the insulin signalling has pointed out the occurrence of an essential set of genes for the insulin action at cellular level. The most important candidate genes are IR, the insulin receptor substrates 1 and 2 (IRS-1, IRS-2) and the phosphatidylinositol 3-kinase (PI3K). In a tissue-specific manner, it has been proposed both PEPCK and glucose-6-phosphatase in the liver, the glycogen-synthase in the liver and in the muscle tissue, the glucose transporter GLUT4 characteristic from skeletal muscle tissue, heart muscle tissue and both white and brown adipose tissues (tissues that require insulin action for glucose uptake) and, finally, the glucokinase and IRS-2 in both the liver and in the β-cell (insulin-independent glucose uptake tissues). All of them are susceptible to conferring insulin resistance (insulin resistance candidate genes), IR and IRS-1 genes being of special clinical significance (Biddinger & Kahn 2006). The lack of IR generates insulin resistance. In fact, it has been described patients with insulin resistance because of lacking of IRs in relationship with lipoatrophic diabetes and type A acantosis nigricans, of low incidence. The IR is a polymorphic gene in humans. Several mutations have been described in the IR gene; it was mapped to the distal short arm of chromosome 19. Several mutations can be silent mutations or affect the reading frame of alpha or beta IR chains. In addition, two point mutations in two different loci of IR have been described (Desbois-Mouthon et al. 1996). Overall mutations in the IR in NIDDM represent 1% of all the cases described in the scientific literature. Therefore, it does not seem to play an essential role in insulin resistance in diabetic patients. Other mechanisms of insulin resistance are related to the possible phosphorylation in serine–threonine residues of IR, which results in hypophosphorylation in tyrosine of IR in response to insulin. Finally, an activation of different tyrosine phosphatases, such as protein-tyrosine phosphatase 1B (PTP1B) or LAR-PTP, or an inactivation of IR/IRS-associated serine-threonine phosphatases, will result in a marked insulin resistance (Roth 1994).

**Mouse models for the study of type 2 diabetes**

Over the last years, using the Cre-LoxP technology, knockout mice have been generated to define the specific role of the IR in insulin target tissues. In the liver, the lack of IR in a tissue-specific manner (LIRKO mice) induced a severe insulin resistance, which resulted in an impaired glucose tolerance (IGT), hyperinsulinemia and hyperglycaemia related to an increased hepatic glucose production. Manifest diabetic phenotype was apparent at 2 months. However, this phenotype ameliorated throughout 2–4 months, and at 4 months, a normoglycaemic metabolism was restored (Michael et al. 2000). The regression of glucose intolerance was paralleled to an increase in glucose consumption by the liver, likely as a result of liver dysfunction. Generation of IR deletion in the liver in an inducible manner (iLIRKO) shows a progressive insulin resistance and glucose intolerance without liver damage. Thus, our data with iLIRKO mice demonstrate that hepatic insulin resistance is critical in triggering the progression to uncontrolled type 2 diabetes (Escribano...
et al. 2009). In the skeletal muscle, the lack of IR in a specific fashion (MIRKO mice) strikingly gave rise to normal insulin and glucose tolerance because of compensatory mechanisms. Thus, the absence of glucose uptake by the skeletal muscle at fed state led to increased glucose consumption by the adipose tissue, with the outcome of overweight and obesity (Bruning et al. 1998). These data contrast with those obtained in the skeletal muscle-specific insulin growth factor 1 receptor (IGF-1R) dominant negative transgenic mice, which, at 6 weeks, were insulin resistant and frankly diabetic. One possible explanation for this discrepancy may be that overexpression of IGF-1R dominant negative might affect both IR and IGF-1R signalling, and the latter signalling remained intact in MIRKO mice (LeRoith & Gavrilova 2006). Specific deletion in white and brown adipose tissues showed low fat mass (LeRoith & Gavrilova 2006). Specific deletion in brown adipose tissue (BAT-IRKO) showed an insulin secretion defect in diabetic mice, without insulin resistance. Thus, acute insulin secretion in response to glucose (IST) and insulin secretion in response to 16.5 mm glucose in isolated islets were much affected (Guerra et al. 2001). Recent data pointed out that those BATIRKO mice showed upregulation of UCP-2 in the β-cells as part of an overall compensatory mechanism. Thus, uncoupling mitochondrial respiratory chain would result in lowering cellular ATP and in an insulin secretion defect (MB, AG, unpublished data). In the pancreatic β-cells, the lack of IR in a tissue-specific manner created an insulin secretion defect, which resulted in a progressive glucose intolerance and hyperglycaemia (BIRKO mice). This diabetic phenotype was related to an impaired acute insulin secretion in response to glucose in fed mice, which is a characteristic feature observed in patients with type 2 diabetes (Kulkarni et al. 1999). Later on, two groups of mutant mice have been defined on basis of responses to intraperitoneal glucose, either diabetic or glucose-tolerant BIRKO mice. Total insulin content was severely reduced in pancreata of BIRKO mice compared with controls. In addition, the β-cell mass and the islet number were severely decreased in mutant mice compared with controls. However, insulin secretion in isolated pancreas in response to secretagogues was more markedly reduced in diabetic than in non-diabetic mutant mice. Gene expression analysis revealed a significant reduction in GLUT2 and glucokinase in both diabetic and non-diabetic BIRKO mice. Taken together, these data primarily indicate the direct role played by the IR in the β-cell growth and, secondly, its role in the onset of the glucose sensing machinery involved in insulin secretion. Recent data obtained in LIRKO mice showed that IR is critical in triggering β-cell hyperplasia in response to hepatic insulin resistance (Okada et al. 2007). In addition, data obtained in iLIRKO mice suggest that IRA isoform rather than IRB isoform is critical in triggering β-cell hyperplasia (unpublished data). The lack of IGF-IR in a β-cell-specific manner also created an insulin secretion defect, related to a reduced expression of GLUT2 and glucokinase in the beta islets, resulting in a defective glucose-stimulated insulin secretion and IGT. However, no effect was observed with regard to β-cell mass and islet number in mutant mice compared with controls. These data showed that IGF-IR is not essential for β-cell growth and development, but participates in the differentiation of glucose-stimulated insulin secretion function by pancreatic β-cells (Kulkarni et al. 2002).

The essential role of the IRS proteins in the insulin signalling cascade has been established during the last 15 years by generating genetic models in mice. In this regard, IRS-1-deficient (IRS-1-/-) mice were growth retarded and mildly insulin resistant, but do not develop diabetes. Rather, these mice developed β-cell hyperplasia resulting in a sustained lifelong compensatory insulin secretion (Araki et al. 1994, Tamemoto et al. 1994). The remnant insulin action in IRS-1-/- mice led to the discovery of IRS-2 as an alternative signalling protein (Sun et al. 1995, Tohe et al. 1995). Previous experiments performed in peripheral tissues of IRS-1-/- mice had suggested that IRS-2 could be a major player of hepatic insulin action (Yamauchi et al. 1996). Thus, IRS-1 works on the metabolism by regulating insulin signals in muscle and adipose tissues, whereas it plays a minor role in the liver. However, IRS-2 can compensate IRS-1 deficiency more effectively in liver and β-cells rather than in muscle or adipose tissues (Kahn et al. 2000). IRS-2-/- mice, by 10 weeks in males and by 23 weeks in females, developed diabetes mellitus primarily because of a severe β-cell failure, suggesting a crucial role of IRS-2 in the growth and survival of pancreatic beta islets (Withers et al. 1998, 1999). However, male IRS-2-deficient mice were transiently hyperinsulinaemic before 10 weeks, suggesting an early insulin resistance (Lee & White 2004). IRS-2, in fact, engages a diverging signalling pathways leading to the activation of PI3K and MAPK. Downstream PI3K, the generation of PIP3 seems to be critical in activating PDK1 and AKT in neonatal hepatocytes, a GLUT2/glucokinase glucose-responding cell as β-cell. Thus, IRS-2-deficient neonatal hepatocytes lack PIP3 generation and AKT activation in response to insulin. Failure of activation of AKT led to the insulin signal impairment on GSK3b, Foxo1 and BAD (Valverde et al. 2003a). In fact, PDX-1 expression is reduced in β-cells from IRS-2 knockout mice (Kushner et al. 2000). PDX-1 expression
and function might be linked to IRS-2 through the Foxo1 (Kitamura et al. 2002). More importantly, PDX-1 haploinsufficiency limits the compensatory islet hyperplasia that occurs in response to insulin resistance in IR/IRS-1 double heterozygous and LIRKO mice (Kulkarni et al. 2004).

**Cellular models for the study of insulin sensitivity and resistance**

A complete understanding of insulin action requires the identification of intracellular pathways that regulate insulin-stimulated growth, development and metabolism. Moreover, mouse models described above lacking key genes involved in the insulin action in a tissue-specific manner have proven to be very useful in understanding the complex insulin signalling network. Accordingly, others and we have developed several immortalized cell lines derived from brown adipose tissue, liver and β-cells lacking insulin resistance or sensitive candidate genes such as IRS-1, IRS-2, IRS-3, IR and PTP1B. These cell lines are unique tools to delineate the tissue specificity of those genes in insulin action or inaction.

**Essential role of IRS-1 in insulin action in brown adipocytes**

The fact that IRS-1-deficient mice showed a phenotype of peripheral insulin resistance (mainly in muscle and white adipose tissue) (Araki et al. 1994, Tamemoto et al. 1994) prompted us to explore IRS-1 signalling as a possible molecular mechanism responsible for insulin-induced lipogenic and thermogenic gene expression in BAT. For that goal, we generated IRS-1-deficient brown adipocytes derived from the corresponding knockout mice. Our results suggest an interesting divergence in signalling pathways downstream of PI3K. While the lack of IRS-1 is not compensated by IRS-2 in the activation of AKT, IRS-2 may substitute in activating P70S6K. However, it was not excluded the possibility of the existence of a PI3K-independent pathway in activating p70S6K as described by Somwar et al. (1998). These results provide consistent evidence indicating that lack of IRS-1 leads to insulin resistance in brown adipocytes at the level of lipogenic gene expression and lipid synthesis in response to insulin (Valverde et al. 1999, Fasshauer et al. 2001). In addition to its essential role in adipogenesis, IRS-1 is a key molecule in mediating insulin-induced thermogenic gene expression in foetal brown adipocytes (Tseng et al. 2004).

IRS-3 is the member of IRS family expressed mainly in white adipose tissue in rodents, where it is detected at levels comparable with IRS-1 (Lavan et al. 1997). Conversely, there are recent evidences indicating the lack of functional gene or protein in human adipocytes (Bjornholm et al. 2002). Our data (Arribas et al. 2003a) suggest that IRS-3 is capable of triggering the lipogenic-related gene expression in brown adipocytes, which relies on IRS-3/p85a/PI3K signalling to achieve transcriptional activation. Finally, foetal brown adipocytes lacking IRS-2 are able to trigger adipogenesis under a specific differentiation protocol; however, they fail to induce glucose uptake in response to insulin. Exogenous expression of IRS-3 signalling restored glucose uptake in response to insulin in IRS-2-deficient differentiated brown adipocytes. More importantly, IRS-3, mainly localized in the cavelin fraction, signals through PI3K/ PKCζ rather than through PI3K/AKT (Arribas et al. 2003b, Escribano et al. 2007). In conclusion, given that neither IRS-2 nor IRS-3 can fully reconstitute insulin action in IRS-1-deficient brown adipocytes, our studies thoroughly implicate a unique function for IRS-1 in BAT.

**PTP1B and IRS-2 play a major role in insulin action and inaction in hepatocytes**

Cellular protein-tyrosine phosphatases (PTPases) play a crucial role in maintaining the steady-state phosphotyrosine content of proteins in the insulin action pathway (Goldstein et al. 1998). Among them, PTP1B has been extensively shown that is expressed in insulin-sensitive tissues, and also that binds to and dephosphorylate IR (Seely et al. 1996) and more recently IRS-1 (Goldstein et al. 2000). In fact, mice lacking PTP1B show increased insulin sensitivity and loss of adiposity, and become hypoglycaemic (Elchely et al. 1998, Klaman et al. 2000). Thus, PTP1B is an important negative regulator of insulin signalling. In fact, recent data from studies on neonatal hepatocytes show that the lack of PTP1B prolonged insulin action; however, insulin sensitivity remained unchanged at that stage. Yet, the lack of PTP1B in adult hepatocytes increased insulin sensitivity. Thus, hepatic insulin sensitivity caused by PTP1B deficiency is acquired through postnatal development (González-Rodríguez et al. 2007). Thus, the lack of PTP1B increased the net free intrahepatic glucose levels in vivo and the glucose uptake in neonatal hepatocytes, which may account for the hypoglycaemic phenotype observed in the knockout mice (Gonzalez-Rodriguez et al. 2008).

Hepatic insulin resistance is a common feature of animal models of insulin resistance and type 2 diabetes. IRS-2 knockout is a genetic mouse model with increased HGP (Kubota et al. 2000). In addition, ablation of IRS-2 results in an impaired development of pancreatic β-cells because of a lack of IGF-I signalling (Withers et al. 1998). However, the combined defect of peripheral insulin resistance and β-cell failure has complicated...
the analysis of hepatic insulin resistance as a primary cause of the type 2 diabetic phenotype of IRS-2-deficient mice. To define the role of IRS-2 in the insulin signalling network of hepatocytes, we have generated neonatal hepatocyte lacking IRS-2 (Valverde et al. 2003b). With regard to insulin signalling, our data show that the lack of IRS-2 is not compensated for either by an elevation of IRS-1 protein content or by an increase in its tyrosine phosphorylation. Furthermore, whereas PI3K activity associated with tyrosine-phosphorylated proteins was reduced by 50% in IRS-2−/− hepatocyte cell lines, the IRS-1-associated PI3K was similar to the wild type. More importantly, the generation of plasma membrane PIP3 is severely reduced in the absence of IRS-2. Therefore, the phosphorylation of AKT and GSK-3 in IRS-2−/− hepatocytes upon insulin stimulation was severely reduced compared with the wild type. These results suggest that IRS-2-associated PI3K activity may constitute the main source of PIP3 in hepatocytes, strengthening the essential and specific role of IRS-2 in mediating downstream signalling to PI3K in the liver.

The alterations in insulin signalling related to the lack of IRS-2 in hepatocytes have important metabolic implications. Insulin fails to activate GS in vitro in IRS-2−/− hepatocytes, a response that can be recovered after IRS-2 reconstitution. In the light of these results, an attractive therapeutic approach to ameliorate hepatic insulin resistance in the absence of IRS-2 would be the use of novel GSK-3 inhibitors, which could improve glucose disposal through the enhancement of glycogen synthesis in the liver (Cline et al. 2002).

The development of fasting hyperglycaemia in type 2 diabetes is believed to be secondary to an increased HGP, mainly through an increase in gluconeogenic pathways. The importance of the liver in the maintenance of normoglycaemia has been studied by different approaches. In mice overexpressing PEPCK or G6Pase, insulin is unable to suppress HGP, despite the presence of hyperinsulinaemia (Valera et al. 1994, Trinh et al. 1998). These findings prompted us to investigate whether the alterations in IRS-2/PI3K signalling in IRS-2−/− hepatocytes could account for a dysregulation in gluconeogenic gene expression. In this regard, insulin failed to inhibit dex/cAMP-induced PEPCK and G6Pase mRNAs in primary hepatocytes of IRS-2−/− mice, but not in wild-type or heterozygous mice. Moreover, IRS-2 reconstitution of deficient hepatocytes led to a full recovery of insulin inhibition of gluconeogenic gene expression. Thus, our in vitro results indicate that the metabolic changes in hepatic function in IRS-2-deficient animals (Previs et al. 2000) could be the result of alterations in insulin signalling that produce a dysregulation of gene expression.

The possibility of controlling hepatic glucose utilization and production as a treatment for type 2 diabetes by overexpressing GK in the liver of diabetic mice has been explored previously (Ferre et al. 1996). In the liver of these animals, glycolysis and glycogen synthesis were induced, while gluconeogenesis was blocked, resulting in the normalization of blood glucose levels. Therefore, this approach could be of potential interest in the treatment of hepatic insulin resistance developing in type 2 diabetic patients associated with the lack of IRS-2, and might circumvent the requirement of β-cell compensatory mechanisms. Related to therapies for ameliorate hepatic insulin resistance, Accili and co-workers have proposed that the dominant negative Foxo1 (Δ256) mutant provides a useful reagent to inhibit gluconeogenesis in experimental systems (Nakae et al. 2001). In fact, these authors have demonstrated that Foxo1 haploinsufficiency corrects hepatic insulin resistance in IR−/− diabetic mice (Nakae et al. 2002). In immortalized neonatal hepatocytes, phosphorylation of Foxo1 by AKT relies on IRS-2 signalling and the Δ256Foxo1 dominant negative mutant rescues the insulin resistance observed in IRS-2-deficient hepatocytes, restoring the inhibition of PEPCK and G6Pase expression induced by dex/cAMP in an insulin-independent manner. These results reinforce the hypothesis that dominant negative Foxo1 could be of potential benefit in reducing glucose production in diabetic patients. The current studies taken together with the genotype of the IRS-2 knockout model implicate IRS-2 as the principal mediator of insulin action in the liver and suggest that IRS-2 signalling may represent a major component in the regulation of gluconeogenesis. However, a new insight into the liver-specific insulin action has changed the prevailing model on insulin signalling involved in liver glucose production. Liver-specific deletion of Foxo1 in IRS-1/IRS-2 DKO mice resulted in significant normalization of the DKO-liver transcriptome and partial restoration of the response to fasting and feeding, near normal blood glucose and insulin concentrations. These results demonstrate that constitutively active Foxo1 significantly contributes to hyperglycaemia during severe hepatic insulin resistance, and that the IRS1/2/PI3K/Akt/Foxo1 branch of insulin signalling is largely responsible for hepatic insulin-regulated glucose homeostasis (Xiaocheng et al. 2008, Fig. 1).

**Insulin vs. glucose signalling: a crucial balance in pancreatic β-cells**

Conditional knockout mice have been generated to define the specific roles of the IR and the IGF-I in pancreatic β-cell development (Kulkarni et al. 1999, 2002). Beta-cell-specific IR deficiency (BIRKO mice) causes defective insulin secretion, which leads to progressive glucose intolerance and hyperglycaemia. This diabetic phenotype reflects an impairment of acute
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Figure 1 Role of IRS-2 in insulin signalling cascade in hepatocytes. IRS-2-deficient hepatocytes showed insulin resistance with reference to IRS-2/PI3K signalling. Downstream from PI3K, insulin failed to activate AKT, GSK-3 and PKCζ in the absence of IRS-2. At the nuclear level, the phosphorylation of Foxo1 is also impaired. The lack of this signalling in hepatic cells resulted in the dysregulation of GS activation and the inhibition of gluconeogenic gene expression by insulin.

Insulin secretion in response to glucose in fed mice, which is characteristic of patients with type 2 diabetes (Kulkarni et al. 1999). Total pancreatic insulin content is severely reduced in adult BIRKO mice compared with controls. Moreover, gene expression analysis has revealed a significant reduction in GLUT2 and glucokinase in both diabetic and non-diabetic BIRKO mice. However, reductions in β-cell mass, islet number and insulin secretion are more pronounced in diabetic than in non-diabetic mutant mice (Otani et al. 2004). Taken together, these data implicate a direct role of IR in β-cell growth and suggest that this receptor may also modulate the glucose-sensing machinery required for normal insulin secretion. Interestingly, the β-cell-specific deletion of the IGF-IR creates a phenotype similar to BIRKO mice. These transgenic animals display a defect in glucose-stimulated insulin secretion because of reduced expression of GLUT2 and glucokinase, resulting in IGT. However, no effect on β-cell mass and islet number was noted in mutant mice compared with controls (Kulkarni et al. 2002). These data suggest that IGF-IR is not essential for β-cell growth and development but participates in the mechanisms of glucose-stimulated insulin secretion of pancreatic β-cells.

Although still controversial, it appears that pancreatic β-cells arise from two main sources. New islets can be formed via budding from the pancreatic ductal epithelium (neogenesis) or through the proliferation of existing islets (Bonner-Weir 2000, Dor et al. 2004). Whereas neogenesis occurs during foetal development (Swenne 1992) and the regeneration of adult pancreas (Smith et al. 1991), pancreatic β-cell proliferation has been observed in the late foetal stages and in normal adult pancreatic islet cells (Hugl et al. 1998, Cousin et al. 1999). Of the signals involved in triggering β-cell mitogenesis, IGF-I is one of the best characterized. However, IGF-I-induced β-cell proliferation appears to depend on glucose, which alone is classic β-cell mitogen (Hugl et al. 1998, Cousin et al. 1999, Rhodes 2000). Thus, glucose at low concentrations (5 mM) activates p42/p44 MAPK and p70S6K by differential mechanisms in insulinoma cells; whereas glucose stimulates p42/p44 MAPK in a Ca2+-dependent manner, p70S6K activation was achieved in an ATP-dependent manner (Briaud et al. 2003). At high concentrations (16.5 mM), glucose induced insulin secretion in islets and lines of insulinomas, but this effect required the expression of the transcriptional factor insulin promoter factor-1/pancreatic duodenal homeobox gene (PDX)-1. In fact, mutation of the PDX-1 transactivation domain impaired insulin secretion in response to 16.7 mM glucose in insulinoma-1 β-cells. Interestingly, the expression of PDX-1 in insulinoma-1 β-cells is fourfold higher than in isolated islets; consequently, insulin secretion by these insulinoma-derived β-cells is highly responsive to glucose. However, it remains uncertain whether the effect of glucose on insulinoma proliferation might reflect an underlying insulin secretion. To address this important issue, we generated foetal pancreatic β-cells without the IR (Guillen et al. 2006). These cell lines have allowed us to examine the role of insulin in β-cell proliferation and, conversely, to analyse the glucose signalling component of β-cell mitogenesis in the absence of IR signalling. Our results demonstrate that the IR, independent of glucose signalling, plays an essential role in the regulation of mitogenesis in foetal pancreatic β-cell lines, requiring both PI3K and MEK-1 pathways to activate mTOR/p70S6K fully. Moreover, our cell lines reveal that glucose induces β-cell mitogenesis in the absence of the IR by activating signals, which are not dependent on PI3K, revealing that phosphorylation at Thr389 of p70S6K is necessary and sufficient to mediate mitogenesis in IR-deficient foetal β-cell lines (67; Fig. 2).

Insulin-resistant states are associated with an increase in the β-cell mass and high levels of circulating insulin. Under these conditions, hyperinsulinemia can persist for a long period of time. Ultimately, the β-cells undergo a failure that leads to diabetes. There is evidence that apoptosis is one of the mechanisms underlying the β-cell mass reduction, but the specific molecular processes remain mostly unknown. At this stage, a question arises whether those persistent high levels of circulating insulin may contribute to β-cell damage. To address this important issue, we have submitted β-cells to a prolonged effect of increasing concentrations of insulin (Guilen et al. 2008). We observed that a prolonged effect of insulin in the presence of serum (15–24 h) in glucose-deprived β-cells induced apoptosis. This
Glucose

Stress

MEK-1 in ever, glucose, in a PI 3 kinase-independent manner, induces kinase.
tuberous sclerosis complex; AMPK, AMP-activated protein pathway to mediate foetal
This tissue has an insulin-independent mechanism to
responsible for the highest glucose disposal in the body.
activation of PI3K, AKT and protein kinase C isoforms
is accomplished by activation of the insulin intracellular
muscle (skeletal muscle and heart) and fat (white and brown), and in suppressing hepatic glucose production. The clearance of circulating glucose in these organs depends on insulin-stimulated translocation of glucose transporter (GLUT4) to the cell surface, which is accomplished by activation of the insulin intracellular signalling cascade, which includes binding to specific IR, tyrosine phosphorylation of IRS proteins and activation of PI3K, AKT and protein kinase C isoforms ζ, λ, α and δ (Huang & Czech 2007). Skeletal muscle is responsible for the highest glucose disposal in the body. This tissue has an insulin-independent mechanism to
increase glucose transport that involves the activation of AMP-activated protein kinase (AMPK) by stimuli such as exercise, hypoxia and ischaemia, although the precise role of AMPK in exercise-induced glucose uptake is still debated (Fujii et al. 2006). The AKT substrate of 160 kDa (AS160) has emerged recently as a point of convergence for both effectors of glucose transport and seems to modulate GLUT4 trafficking (Fujii et al. 2006). Even though the GLUT4 protein content is normal in the muscle tissue from subjects with type 2 diabetes, the capacity of insulin to stimulate translocation of GLUT4 to the plasma membrane is impaired. In contrast to the effect of insulin, contraction-stimulated glucose uptake and GLUT4, translocation in diabetic patients is normal, providing evidence that exercise might be able to bypass defects in insulin signalling.

Insulin resistance, which can be defined as a diminished ability of the cell to respond to the action of insulin, is the most important pathophysiological feature in many pre-diabetic states and is the first detectable defect in type 2 diabetes. The pathogenesis of type 2 diabetes involves abnormalities in both insulin action and secretion. Insulin resistance is usually compensated by hyperinsulinaemia. Although moderate hyperinsulinaemia might be tolerated in the short-term, chronic hyperinsulinaemia exacerabates insulin resistance and contributes directly to β-cell failure and diabetes (White 2003). Skeletal muscle, liver and adipose tissue are the three major tissues involved in insulin resistance. However, insulin resistance can originate in adipose tissue, where insulin resistance leads to an increase in lipolysis, with a subsequent release of glycerol and free fatty acids (FFA) into the circulation. It is widely accepted that the lipid accumulation may contribute to the development of skeletal muscle insulin resistance, and that the increased availability and fatty acid utilization may increase hepatic glucose production (White 2003). In fact, the progression of insulin resistance in rats on a high-fat diet is closely related to plasma FFA (Jiao et al. 2008). Both genetic and environmental factors can contribute to the development of insulin resistance and, in the second group, obesity has been proposed as an important contributor.

Obesity is a risk factor for developing type 2 diabetes, in part because of the fact that adipose tissue expresses and secretes a variety of adipokines such as adiponectin, resistin and leptin, and cytokines such as interleukin (IL)-6, monocyte chemoattractant proteins (MCP)-1 and TNF-α, that can act at both local and systemic levels, modulating insulin sensitivity. Adipose tissue, in particular the visceral compartment, is now recognized as the primary contributor to insulin resistance syndrome. Several factors secreted from the adipose tissue, including pro-inflammatory cytokines and FFA, can impair insulin signalling, altering insulin-mediated

**Figure 2** Insulin vs. glucose signalling in β-cells. Insulin independently of glucose signals through IRS-dependent PI 3 kinase/Akt and MEK-1 pathways in β-IRLoxP β-cells. However, glucose, in a PI 3 kinase-independent manner, induces MEK-1 in β-IR-/-β-cells. Both signalings converge on mTOR/p70S6K pathway to mediate foetal β-cell proliferation. TSC, tuberous sclerosis complex; AMPK, AMP-activated protein kinase.

**β-cell proliferation**

**β-IRLoxP**

Insulin

Glucose

Glucose metabolism

MEK1/2

AMPK

mTOR

p70S6K

**β-IR-/-**

Akt

IRS-1/2

Shc

Grb2

TSC1/2

AMP

Stress

Ser 2448

Thr 421

Ser 423

Thr 389

Ser 2448

The negative impact of obesity on insulin action: role of proinflammatory cytokines in adipose tissue and skeletal muscle

Insulin exerts a dominant role in regulating glucose homeostasis through orchestrated effects on the promotion of glucose uptake in peripheral tissues, such as the muscle (skeletal muscle and heart) and fat (white and brown), and in suppressing hepatic glucose production. The clearance of circulating glucose in these organs depends on insulin-stimulated translocation of glucose transporter (GLUT4) to the cell surface, which is accomplished by activation of the insulin intracellular signalling cascade, which includes binding to specific IR, tyrosine phosphorylation of IRS proteins and activation of PI3K, AKT and protein kinase C isoforms ζ, λ, α and δ (Huang & Czech 2007). Skeletal muscle is responsible for the highest glucose disposal in the body. This tissue has an insulin-independent mechanism to...
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processes including glucose homeostasis and lipid metabolism (Arner 2003). Accordingly, obesity is now considered a chronic state of low-intensity inflammation. In this regard, recent studies reveal that obesity is also associated with an increase in infiltration of adipose tissue with macrophages, which contributes to the inflammatory process through the additional secretion of cytokines (Lumeng et al. 2007). The mechanisms by which adipose tissue recruits and maintains macrophages could involve the expression of MCP-1 and intercellular adhesion molecule-1. Recent studies revealed that those subjects with the highest transcription rates of genes encoding TNF-α and IL-6 seemed prone to developing obesity, insulin resistance and type 2 diabetes (Fernandez-Real & Pickup 2008). Accordingly, we here review the impact of these cytokines in modulating insulin action on glucose transport in adipose tissues and skeletal muscle.

At the molecular level, insulin resistance correlates with impaired insulin signalling in peripheral tissues. From the complex intracellular signalling pathways activated by insulin, the tyrosine phosphorylation of IRS-1–4 proteins is a crucial event in mediating insulin action. This step is one of the key molecular events in inflammation-associated insulin resistance. The mechanisms affecting IRSs involve proteasome-mediated degradation, phosphatase-mediated dephosphorylation and serine phosphorylation of IRS-1, which converts IRS-1 into an inhibitor of the IR tyrosine kinase activity, as reviewed previously (Pirola et al. 2004). Several stress and pro-inflammatory kinases as well as more recent players, such as tyrosine-phosphatases, seem to be involved in the molecular mechanisms by which TNF-α and IL-6 disrupt IRSs signalling, as we will further explore. Pharmacological and genetic approaches to overcome cytokine-induced insulin resistance will also be discussed in this review.

The role of TNF-α in insulin resistance in skeletal muscle: contribution of kinases and phosphatases

TNF-α has been proposed as a link between adiposity and the development of insulin resistance because a majority of type 2 diabetic subjects are obese, TNF-α is highly expressed in adipose tissues from obese subjects (Kern et al. 2001), and because obese mice lacking either TNF-α or its receptors show protection from developing insulin resistance (Hotamisligil 2003). Rather than acting systemically, TNF-α seems to act locally at the site of adipose tissue through autocrine or paracrine mechanisms, affecting insulin resistance and inducing IL-6 (Briaud et al. 2003). Circulating levels of soluble TNF-α receptors now seem well correlated with BMI, and impairment in TNF-α processing can improve systemic insulin sensitivity (Serino et al. 2007). On the other hand, TNF-α has lipolytic and antiadipogenic effects on white and brown adipose tissues (Arner 2003, Valverde et al. 2005). This paradox could be due to proliferative and anti-apoptotic effects of this cytokine in the obese adipocyte, and could be mediated by the differential expression of its soluble and membrane-anchored receptors. Moreover, TNF-α blocks skeletal muscle differentiation, causes sarcopenia and produces insulin resistance in the skeletal muscle of healthy humans (Plomgaard et al. 2005).

Both ceramides and FFA were reported to induce insulin resistance in peripheral tissues and production of these molecules could be the consequence of activation of sphingomyelinase or lipolysis by TNF-α (Arner 2003). TNF-α-deficient mice exhibit lower circulating FFA and triglycerides than wild-type animals (Uysal et al. 1997). Ceramide production is activated by TNF-α in brown adipocytes and exogenously added C2-ceramide inhibits AKT activity throughout ceramide-activated protein-phosphatase 2A (Teruel et al. 2001). Furthermore, infusion of TNF-α in rodents leads to impairment of insulin-stimulated skeletal muscle glucose uptake (Nieto-Vazquez et al. 2007). Accordingly, neutralization of TNF-α with specific antibodies has the opposite effect and improves insulin resistance in rats (Hotamisligil et al. 1994). In contrast to the findings in rodent models, however, TNF-α neutralization has no beneficial effect in terms of insulin sensitivity in humans (Bernstein et al. 2006). Variations in TNF-α genotypes in the mediation of the TNF-α action could explain these different effects (Fontaine-Bisson et al. 2007).

The interaction between TNF-α and insulin signalling is most important for local insulin resistance in obesity. When cells are directly exposed to TNF-α, this adipokine inhibits insulin signalling by affecting IRS proteins (Hotamisligil 2003). Stress kinases and inflammatory pathways that are activated in response to TNF-α, such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), have been proposed as mediating TNF-α serine phosphorylation of IRS-1 in human adipocytes and skeletal muscle cells (Bouzakri & Zierath 2007). In this regard, ablation of Jnk1 decreases the development of insulin resistance associated with dietary obesity. Furthermore, ERK and p38MAPKs could inhibit insulin signalling by TNF-α at the level of IRS-1 and IRS-2 in 3T3-L1 adipocytes, whereas JNK could mediate the feedback inhibitory effect of insulin (White 2003, Pirola et al. 2004). Activation of ERK and p38MAPK by TNF-α is involved in this impairment of normal tyrosine phosphorylation by insulin of IRS-2 in brown adipocytes (Teruel et al. 2001, Hernandez et al. 2004). Chronic exposure to TNF-α induces a state of insulin resistance on GLUT4 translocation to the plasma membrane in murine primary myotubes (de Alvaro
et al. 2004), in accordance with the effect produced in the muscle in vivo, and systemically (Hotamisligil et al. 1994). The Ser307 residue of IRS-1 seems to be one of the residues phosphorylated by TNF-α via activation of the beta isomor of p38MAPK. Moreover, activation of inhibitor kappa B kinase (IKK)/β, dependent on the functionality of p38MAPK, was observed during chronic treatment with TNF-α in murine myotubes (de Alvaro et al. 2004). Then, IKK/β could act either downstream of p38MAPK or directly and mediate TNF-α-induced serine phosphorylation of IRS-1. Accordingly, IKK/β inhibition with salicylate or targeted disruption of ikkβ reversed obesity and diet-induced insulin resistance (Gao et al. 2003, de Alvaro et al. 2004). In this regard, the glucose-lowering effects of the anti-inflammatory compounds, salicylate and its derivative aspirin, were identified more than 100 years ago. However, the positive effects of high-dose aspirin are limited by its toxicity effects on the gastrointestinal tract, as reviewed previously (De & Olefsky 2008).

On the other hand, the insulin signalling cascade is negatively regulated by protein phosphatases such as the PTP1B, which dephosphorylates the phosphotyrosine residues of the IR and IRS-1. The expression and activity of PTP1B have been found to be increased in the muscle of diabetic and obese humans and rodents (Klaman et al. 2000, Delibegovic et al. 2007). Moreover, non-coding polymorphisms in the PTP1B gene have been found in various populations, displaying increased phosphatase muscle expression and association with insulin resistance (Bento et al. 2004). In this regard, transgenic overexpression of ptp1b in the muscle causes insulin resistance, showing impaired insulin signalling and decreased glucose uptake in this tissue (Zabolotny et al. 2004). By contrast, mice lacking PTP1B (either in total body or in skeletal muscle) exhibit increased insulin sensitivity, resistance to weight gain on a high-fat diet and an increased basal metabolic rate (Elchebly et al. 1998, Klaman et al. 2000, Delibegovic et al. 2007). Furthermore, PTP1B deficiency also reduces the diabetic phenotype in mice with polygenic insulin resistance (Xue et al. 2007) and treatment with PTP1B antisense oligonucleotide improves insulin sensitivity in db/db mice (Gum et al. 2003). Accordingly, modulation of genes such as PTP1B might also contribute to the pathogenesis of TNF-α-induced insulin resistance. In this regard, we described for the first time that brown adipocytes treated with TNF-α showed significant enhancement of PTP1B expression and activity, and the lack of ptp1b in these cells conferred protection against TNF-α-induced insulin resistance on glucose uptake and insulin signalling (Fernandez-Veledo et al. 2006a). Additionally, the expression of PTP1B was also found to be upregulated by TNF-α in murine myoblasts and in adipose tissue and muscle (Nieto-Vazquez et al. 2007, Zabolotny et al. 2008). More importantly, chronic exposure to TNF-α does not induce insulin resistance either on glucose uptake or on insulin signalling in PTP1B-deficient myocytes (Nieto-Vazquez et al. 2007). Moreover, PTP1B+/− mice showed complete protection against TNF-α-induced systemic insulin resistance and glucose intolerance during glucose and insulin tolerance tests (Nieto-Vazquez et al. 2007). Then, the lack of PTP1B expression confers protection against TNF-α-induced insulin resistance in the skeletal muscle both in vitro and in vivo (Nieto-Vazquez et al. 2007). Therefore, TNF-α impairs the action of insulin in myocytes at the level of IRS-1 by a double mechanism that involves (i) serine phosphorylation by IKK and p38MAPK at the Ser307 residue and (ii) tyrosine dephosphorylation by PTP1B. Accordingly, inhibition of IKK activation with salicylate and ablation of PTP1B restore insulin sensitivity in myocytes in the presence of the cytokine, as summarized in Figure 3. In this regard, new mono- and disalicyclic acid derivates

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**Figure 3** Molecular mechanisms by which TNF-alpha induced insulin resistance in the skeletal muscle. Treatment with TNF-α impairs insulin-stimulated glucose uptake in the skeletal muscle at the level of the IRS-1 by a double mechanism that involves (i) serine phosphorylation by IKK and p38MAPK and (ii) tyrosine dephosphorylation by the phosphatase PTP1B. Inhibition of IKK activation with salicylate, inhibition of p38MAPK with PD169316 or genetic ablation of PTP1B restores insulin sensitivity in the presence of the cytokine.
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have been used very recently as PTP1B inhibitors and potential anti-obesity drugs (Shrestha et al. 2007).

In addition to these signalling impairments, long-term effects of TNF-α on gene expression take place. These effects are mediated via stimulation or repression of the expression of many genes responsible for glucose and FFA uptake and storage. For example, TNF-α has been shown to downregulate the genes for adiponectin, GLUT4, IRS-1, C/EBPz, PPARγ and perilipin in adipocytes, involving the transcription factor NF-κB (Bento et al. 2004). Moreover, the adipokines repressed GLUT4 gene expression in brown adipocytes by interfering with C/EBPz accumulation (Ruan et al. 2002).

Molecular mechanisms involved in the modulation of insulin action by IL-6 in the skeletal muscle

Interleukin-6 has been described as a pro-inflammatory cytokine that can contribute to insulin resistance in peripheral tissues when overproduced by the adipose tissue (Kern et al. 2001). In fact, elevated plasma concentrations of IL-6 are detected in obese and insulin-resistant patients, and a chronic elevation of IL-6 is not desirable as it may compromise insulin sensitivity. Furthermore, a single polymorphism in the IL-6 gene promoter has been linked to reduced insulin sensitivity and type 2 diabetes (Fernandez-Veledo et al. 2006b). On the other hand, the skeletal muscle also secretes IL-6, particularly after exercise, with positive metabolic effects that can modulate insulin action (Fernandez-Real et al. 2000, Penkowa et al. 2003). In this regard, an impaired ability to exercise and to oxidize fatty acids was observed in the IL-6 knockout mice at 3 months of age, and by 9 months of age, these mice were obese and insulin intolerant (Carey et al. 2006). The role of IL-6 seems to be anti-inflammatory in such physiological situations. However, a recent study that describes reduced body weight under chronically elevated IL-6 levels also shows impaired insulin-stimulated glucose uptake by the skeletal muscles of these mice (Wallenius et al. 2002), indicating divergent opinions on the impact of IL-6 on insulin sensitivity.

So far, additional data regarding the role of this cytokine in the regulation of metabolism are highly controversial (Franckhauser et al. 2008). Pre-treatment with IL-6 in vivo blunted insulin’s ability to suppress hepatic glucose production and to stimulate glucose uptake in the skeletal muscle (Kristiansen & Mandrup-Poulsen 2005). However, other studies reported a lack of effect or a positive effect of IL-6 on whole body glucose disposal in rats and humans respectively (Carey et al. 2006). Alternatively, IL-6 induced insulin resistance in hepatocytes, adipocytes and myocytes (Kim et al. 2004). In addition, palmitate-induced IL-6 production led to the inhibition of insulin-stimulated glucose uptake in myocytes as demonstrated by the prevention of these effects with anti-IL-6 or anti-Toll-like receptor-2 antibodies (Rieusset et al. 2004).

Accordingly, when we evaluated the impact of IL-6 treatment on myocytes and skeletal muscle, a dual effect on insulin action was observed: additive at short-term and negative after chronic treatment. IL-6 per se activated glucose uptake because of the sequential phosphorylation of LKB1/AMPK/AS160 pathway in myotubes (Jove et al. 2006), while diminished AMPK activity was found in the muscle from IL-6 knockout mice (Nieto-Vazquez et al. 2008). Short-term treatment with IL-6 improved insulin stimulation of glucose uptake in myocytes, because of the activation of AMPK and AKT by IL-6 and insulin, respectively, and was additive on AS160 phosphorylation (Ruderman et al. 2006). Moreover, an improvement in glucose and insulin tolerance tests was observed in mice treated for 3 h with IL-6. This situation can mimic the positive effect of IL-6 on insulin sensitivity when released from the muscle after exercise, as schematized in Figure 4. Chronic exposure to IL-6 impaired insulin-stimulated GLUT4 translocation and insulin signalling in both myotubes and skeletal muscle, and caused systemic insulin resistance as observed from glucose and insulin tolerance tests. This situation imitates the chronic elevation of IL-6 that causes insulin resistance when secreted by the adipose tissue in obesity. This dual behaviour of IL-6 has been previously observed in human skeletal muscle cells (Al Khalili et al. 2006).

The molecular mechanism underlying IL-6-mediated insulin resistance involves activation of pro-inflammatory kinases, suppressor of cytokine signalling (SOCS) and phosphatases that converge at the IRS-1 level. Along these lines, IL-6 impairs insulin signalling in murine myoblasts at the level of IRS-1 by three mechanisms that involve (i) serine-phosphorylation by JNK, (ii) impairment of tyrosine phosphorylation by SOCS3 and (iii) tyrosine dephosphorylation by PTP1B (Fig. 4). Accordingly, the deficiency in PTP1B confers protection against IL-6-induced insulin resistance in the skeletal muscle either in vitro or in vivo, in accordance with the protection against systemic insulin resistance observed in mice.

Although glucose uptake in body fat only accounts for 20% of the uptake in the whole body, insulin resistance in the adipose tissue appears to be an early and pivotal phenomenon in the development of type 2 diabetes. When insulin sensitivity based on glucose and lipid metabolism was explored through human adipocyte differentiation, an inverse biphasic response was observed: an increase in insulin sensitivity in cells differentiated for 14 days and an insulin-resistant state in prolonged cultures. Thus, long-term treatment with insulin impaired GLUT4 translocation to the plasma...
membrane and insulin signalling at IRS-1/AKT level (Fernandez-Veledo et al. 2008). This *in vitro* situation might imitate the chronic elevation of insulin during insulin-resistant states observed in humans. Furthermore, adipocytes release different adipokines through the differentiation process. Thus, adiponectin secretion was in keeping with insulin sensitivity through adipogenesis, in accordance with the inverse correlation with insulin-resistant states described. In contrast, secretion of pro-inflammatory cytokines, such as MCP-1 and IL-6, as well as FFA release, were markedly stimulated in human adipocytes differentiated for 21 days, in accordance with elevated plasma concentrations of these factors detected in obese and diabetic patients. Moreover, adipocyte-conditioned media modulate insulin sensitivity in human skeletal muscle by producing an inverse biphasic response. Thus, signals coming from undifferentiated adipocytes enhanced insulin-induced glucose uptake and AKT phosphorylation in muscle cells; whereas signals from more differentiated adipocytes induced insulin resistance, although insulin resistance was detected earlier than in adipocytes (Fernandez-Veledo et al. 2008). This behaviour mimics hyperinsulinaemia because insulin action is restored when adipocytes are cultured in the absence of the hormone, with a complete downregulation of IL-6 levels. These data point out the important role of this cytokine in inducing insulin resistance in both skeletal muscle and adipose tissue when overproduced by adipose tissue.

**Summary**

Insulin resistance is the most important pathophysiological feature in many pre-diabetic states. Type 2 diabetes mellitus is a complex metabolic disease and its pathogenesis involves abnormalities in both peripheral insulin action and insulin secretion by pancreatic β-cells. The creation of monogenic or polygenic genetically manipulated mouse models regarding insulin target genes in a tissue-specific manner it was of great help to elucidate the tissue specificity of insulin action and its contribution to the overall insulin resistance. However, a complete understanding of insulin action requires the identification of intracellular pathways that regulate insulin-stimulated growth, differentiation and metabolism. Accordingly, cell lines derived from brown adipose tissue, liver and β-cells lacking insulin resistance or sensitive candidate genes such as IRS-1, IRS-2, IRS-3, IR and PTP1B have been developed. Obesity is a risk factor for developing several components of the metabolic syndromes such as type 2 diabetes, dyslipidaemia and systolic hypertension, mostly because brown and white adipose tissues express and secrete a variety of adipocytokines that can act at both local and systemic levels, modulating insulin sensitivity. Recent studies...
revealed that those subjects with the highest transcription rates of genes encoding TNF-α and IL-6 seemed prone to developing obesity, insulin resistance and type 2 diabetes. Accordingly, we specifically focus this review on the impact of those adipocytokines on the modulation of insulin action and resistance in the skeletal muscle. However, in the last years, there is evidence for the presence of physiologically significant BAT in adult humans (Cypess et al. 2009). Most importantly, the amount of brown adipose tissue is inversely correlated with the body mass index, especially in older people. Thus, effort has to be put in to elucidate the role played by brown fat in the pathogenesis of human obesity and to implement new strategies focused on restoring its functionality in obese individuals. New mouse models challenging brown adipose tissue functionality or alternatively inducing its hyper trophy will help understand its contribution to the body weight regulation.

Conflict of interest

The author declares no conflict of interests.

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